PLANT BREEDING

Principles and Methods

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To
Nikhil
Nishith and Pushpa

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Foreword

The contributions made by Indian plant breeders during the last three decades are well known to the outside world. It may be favourably compared with achievements of plant breeders in any other country. But the work of Indian plant breeders has not been compiled at one place so that students of plant breeding in India may have an easy access to it. Indian students hardly find a suitable textbook on plant breeding except those written by Allard ; Elliot ; Briggs and Knowels ; Hayes, Immer and Smith ; and Simmonds, which were never written for Indian conditions. Only two text books relate particularly to the Indian context : these are 'Breeding of Asian Field Crops' by Poehlman and Borthakur and 'Elementary Principles of Plant Breeding' By Chaudhari. The first work gives an excellent cropwise treatment of breeding methods used and salient achievements but it does not deal with the principles and the breeding methods to an appreciable extent. The book by Chaudhary does not touch on several relevant topics, e.g., population improvement in cross-pollinated crops, polyploidy in plant breeding and distant hybridization in crop improvement.

The present book, 'Plant Breeding : Principles and Methods' by Dr. B.D. Singh, Professor in the Department of Genetics and Plant Breeding of this Institute is a welcome effort. He has attempted to write a text book in the real sense with adequate emphasis on principles as well as on methods of plant breeding. The beauty of this book is that the examples are taken from the Indian conditions. He has particularly devoted a chapter to 'Organisations for Crop Improvement in India' which also outlines the history of agricultural research and development in the country. In addition, a chapter on 'In Vitro Methods' highlighting the possibilities offered by this novel technique adds tremendous value to the book. Students of plant breeding in India will definitely find this book a real help in getting the background of the work done in the past and the future projections of the subject. As such, this book bridges the gap of not having a suitable text book in plant breeding for Indian conditions. The language of the entire book is such a simple and easy one that even the complicated topics of the subject will be understood well by the readers. The figures and drawings used in the book make the text more easily understandable.

I personally congratulate Dr. B.D. Singh for his maiden attempt at writing such a valuable textbook. At the same time, I am confident that this book will be widely accepted in the universities, colleges and research institutions engaged in evolution of crop varieties.

1983

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Preface

(Fourth Edition)

I feel exhilarated in writing the preface for the fourth edition of *Plant Breeding : Principles and Methods.* Many additions have been made in this edition, *e.g.*, a new chapter 'Effectiveness of the breeding methods for self-pollinated crops', a thorough revision of the chapter 'Quality seed : classes, production practices and maintenance', inclusion of 'doubled haploid technique in the chapter' 'other approaches to breeding of self-pollinated crops' etc. These additions, it is hoped, will enhance the usefulness of the book.

In the third edition of the book, two new chapters, *viz.*, 'Breeding for insect resistance' and 'Release of new varieties', were included. These chapters contained many errors, which I have attempted to remove in this edition. The details of the procedures for the testing of new strains, more particularly the zoning patterns, are still in a flux, and are expected to remain so for some time in the future.

I have benefited from discussions with many students and teacher colleagues who provided constructive and well-intended criticisms as well as suggestions for new inclusions. I am grateful to Late Dr. G.S. Sharma, Varanasi, for providing the brasic information for the chapter 'Release of new varieties', and to Dr. P. Singh, Nagpur, for having prepared the initial draft for the chapters 'Biometrical techniques in plant breeding' and 'Breeding for insect resistance', and for several other contributions and suggestions for improvement. Dr. Harihar Ram, Pantnagar, was kind to go through and comment on 'Release of new varieties', while the comments of Dr. D.P. Singh, Faizabad, provided the basis for a thorough revision of the draft on 'Effectiveness of the breeding methods for self-pollinated crops'. Thanks are also due to Dr. B.S. Manake, Dhule, Dr. R.K. Raghuvanshi, Jaipur, Dr. L. Krishnamurthy, ICRISAT, Hyderabad, Dr. G.S. Gagtap, Khargone, M.P. and Mr. A.K. Singh for their valuable suggestions.

I am aware that many further improvements are due in the book in view of the very rapid accumulation of new findings. It is this challenge of a book from going obsolete which provides the ultimate thrill to an author.

1990 **B.D. Singh**

Varanasi

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Preface

(1st Edition)

The present text book has been prepared for B.Sc. (Ag.) students of Indian universities. An attempt has been made to present the principles and methods of plant breeding in a simple language and to use suitable examples from the Indian context. Tables and charts have been used, where necessary, to make the text clearer, and it is hoped that this would be of some help to the students. There is a certain amount of repetition throughout the book, which is intentional in the hope that this would act as a reinforcement for the memory.

A text book has to oscillate between two opposite and demading necessities. The *first* necessity is to keep the text easily understandable, which necessitates the use of a simple language and particularly, elaborate exposition. The *second* necessity relates to providing as much uptodate information as possible within the limitations of the space in the text ; this makes it essential to be concise and to use a technical language which is relatively more difficult to comprehend for a beginner. An emphasis on the first need is likely to make the text too elementary, while an emphasis on the second would adversely affect its comprehensibility. The success of an author, therefore, would primarily depend on how well he has been able to achieve an acceptable balance between the two opposing demands. The point of this balance, by its nature, would be highly subjective, but the norm would be the majority view. Whether this balance has been achieved in this text would, therefore, be known only after the reactions of the readers are available.

A text book can hardly claim originality either in content or in presentation. The present text book is no exception and it draws heavily from earlier texts on plant breeding, notably, Principles of Plant Breeding by R.W. Allard, Principles of Crop Improvement by N.W. Simmonds, Breeding Asian Field Crops by J.M. Poehlman and D. Borthakur, Methods of Plant Breeding by H.K. Hayes, F.R. Immer and D.C. Smith and Plant Breeding and Cytogenetics by F.C. Elliot, and from other published works, notably, A History of the Indian Council of Agricultural Research by M.S. Randhawa. Fifty Years of Agricultural Research and Education by ICAR and New Vistas in Crop Yields by ICAR.

In addition, I have received help from my colleagues, Dr. R.M. Singh, Dr. A.K. Richharia, Dr. V.P. Singh and Dr. U.P. Singh who have contributed in one way or the other in the development of this text. I wish to express my appreciation for Dr. Mahatim

Singh, Director, Institute of Agricultural Sciences, Banaras Hindu University. for his keen interest in the project and for some valuable suggestions regarding the manuscript. My students Sri R.P. Singh, Parshotam Singh, H.K. Jaiswal, Vinod Tiwari and P.S. Bhatnagar have contributed in collection of material and correction of the typescript.

My colleagues, students and the literature have contributed to whatever usefulness and quality is present in the text, but the errors, omissions and deficiencies are my own copyright and no one else should be credited for them.

However, all this would not have been possible without my long association with Professor R.B. Singh, Plant Production and Protection Officer, F.A.O., Bangkok, who instilled in me the habit of spending long hours with the work at hand.

In the end, I must express my appreciation for my wife Pushpa and my sons Pintu and Cheenu who have been affectionate, understanding and patient during the long hours I spent on the manuscript, which often were very trying indeed.

B.D. Singh

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CHAPTER 1

Introduction to Plant Breeding

The foodgrain production in India has increased from 54.92 million tonnes (from 99.28 million hectares) in 1949-50 to 150.47 million tonnes (from 127.06 million hectares) in 1985-86. This represents an increase of about 274 per cent during a period of 36 years. As a result, the nation has become almost self-sufficient in foodgrains. But the population in India is growing at an alarming rate of 2.5 per cent per year. This makes it necessary that the foodgrain production should also increase at least at the same rate or even at a faster rate in order to improve the nutritional status of the masses.

This large increase in foodgrain production has resulted from an increase in net cropped area, (127.06 million hectares in 1985-86 as against 99.28 million hectares in 1949-50), increased quantum and better management of inputs, such as fertilizer, irrigation water, plant protection and cultural practices, and from improved crop varieties (Fig. 1.1). It is doubtful that the net cropped area can be increased indefinitely, but there is still some scope through double and multiple cropping. Improved input management practices

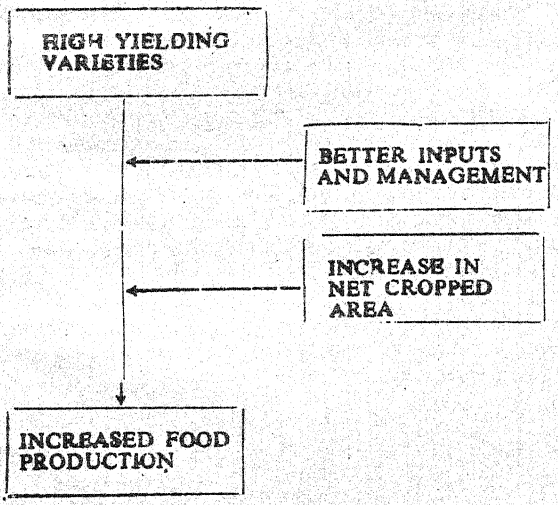


Fig. 1.1. Factors contributing to increased food production in India.

are yet to be fully exploited, and vast tracts of cultivated lands are very poorly managed. In future, agricultural production is most likely to increase from providing better environment through management and from crop varieties capable of fully exploiting the environment provided. Better environment alone cannot lead to better yields from inferior varieties beyond a certain limit. The limit of yield is set by the genetic makeup of the variety. Improving the environment beyond a certain limit for any variety may adversely affect its performance. This point is illustrated by the tall and dwarf varieties of wheat. Tall wheat varieties respond to nitrogen application upto 60 kg/ha, but higher doses of nitrogen reduce yields primarily due to severe lodging. Dwarf wheat varieties, on the other hand, give increased yields upto, or even beyond, 120 kg nitrogen per hectare. It is believed that the genetic makeup of the crop plants is such that it would permit considerable, perhaps endless, changes and improvements. Further, the changes brought about in the present varieties probably represent only a small portion of the possibilities. Thus continuous favourable changes in the genotype of crop varieties are a must for increasing yields from crop plants.

Plant breeding deals with this aspect of crop production : *It consists of the principles and the methods required for favourably changing the genetic constitution of crop plants.* This usually produces crop varieties more suited to human needs in one or more aspects than the existing ones.

HISTORY OF PLANT BREEDING

It is reasonable to assume that plant breeding, howsoever primitive, began when man first chose certain plants for cultivation. *The process of bringing a wild species under human management is referred to as domestication.* There is bound to be some selection during domestication. This is likely to give rise to better types than the wild ones. Thus domestication may be regarded as a method of plant breeding. Domestication continues till today, and is likely to continue for some time in future. This is particularly true in the case of timber trees, medicinal plants, microbes and plants satisfying some special requirements. A remarkable case of domestication in recent years was that of *Penicillium* for penicillin and subsequently of several other fungi for various antibiotics. Transfers of specific genes, *e.g.*, for disease resistances, from wild species to cultivated ones may be regarded as domestication of those genes, *i.e.*, of a part of the genome of wild species.

During the long period of prehistoric and historic cultivation, natural selection has definitely acted on the domesticated species. It is likely that man also exerted some selection, knowingly or unknowingly. Movement of man from one area to another brought about the movement of his cultivated plant species. *The introduction*

into an area of new plant species or varieties from other parts of the world is an integral part of plant breeding today ; this aspect will be covered in a greater detail in Chapter 2.

Babylonians and Assyrians pollinated date palm artificially as early as 700 B.C. In 1717, Thomas Fairchild produced the first artificial hybrid. Joseph Kolreuter, a German, made extensive crosses in tobacco between 1760 and 1766. Knight (1759-1835) was perhaps the first man to use artificial hybridization to develop several new fruit varieties. Le Couteur and Shireff, around 1840, used individual plant selection and progeny test to develop some useful cereal varieties. Vilmorin (1856) further developed the progeny test and used this method successfully in improvement of sugarbeets (*Beta vulgaris*). The individual plant selection method was developed in detail by Nilsson and his associates at Svalof, Sweden around 1900. In 1903, Johannsen proposed the pureline theory that provided the genetic basis for individual plant selection.

The modern plant breeding methods have their basis in the genetic and cytogenetic principles. The science of genetics began with the rediscovery of Mendel's paper in 1900, which was originally published in 1866. Gregor Mendel's laws of inheritance provided the basis for the vast knowledge that has accumulated in genetics. Numerous workers who determined the various modes of inheritance have contributed to the development and understanding of plant breeding. Realisation that the chromosomes are the carriers of genes has led to the development of specialized plant breeding methods for chromosome engineering. A noteworthy development resulted from the studies of G.H. Shull on inbreeding in maize (*Zea mays*). He found that inbreeding produced a considerable loss of vigour. But when some of the weak inbred lines were crossed, the resulting hybrids were more vigorous than the original variety. These observations led to the production of hybrid varieties which are common in the case of maize, jowar (*Sorghum bicolor*), bajra (*Pennisetum americanum*) and several other crops.

NATURE OF PLANT BREEDING

Science is the knowledge gathered through scientific methods. The scientific method consists of observation, formulation of a hypothesis, experimentation and conclusion either to accept or to reject the hypothesis. Thus science is objective in approach, while art is highly subjective. Whether plant breeding is an art or a science is hardly a point of dispute anymore. In early days, man depended on his skill in selecting better plants. His knowledge about the plants was very limited. He knew nothing about inheritance of characters, role of environment in producing them and the basis of variation in various plant characteristics. His methods of selection were designed without an understanding of the principles of inheritance. Therefore,

plant breeding then was largely, often exclusively, an art. But the present plant breeding methods are based on scientific principles of plant sciences, particularly of genetics and cytogenetics. Thus, a large part of the breeding work is purely science with very little art involved. However, the selection of desirable plants even today is largely an art since much depends upon the ability of the breeder to identify superior plants by visual observation. The amount of seed set in crosses also depends, to a large degree, on the skill of person making the crosses. Thus plant breeding today is mostly a science. A modern plant breeder has to know all he can about his plants, and that involves knowledge of several related disciplines.

THE DISCIPLINES A BREEDER OUGHT TO KNOW

To be successful, a plant breeder must know all he can about the plants he is working with. Thus he should have an understanding of the following : (1) botany, (2) genetics and cytogenetics, (3) agronomy. (4) plant physiology, (5) plant pathology, (6) entomology, (7) bacteriology, (8) plant biochemistry, and (9) statistics.

Botany. A plant breeder must have a clear understanding of the morphology and reproduction of the plants he aims to improve. He should also be familiar with the taxonomy of the plant.

Genetics and cytogenetics. The principles of genetics and cytogenetics provide the basis for plant breeding methods. Therefore, a thorough knowledge of these subjects is essential for a rapid and efficient improvement of a crop plant.

Agronomy. A good breeder is first a good agronomist. He must be able to raise a good crop in order to select and evaluate his material.

Plant Physiology. Adaptation of a variety is determined by its response to environmental factors like heat, cold, drought, salinity etc. A knowledge of the physiological basis of these responses would help the breeder in developing cold, drought or salinity tolerant varieties. In addition, several physiological approaches to breeding for higher yields are being developed.

Plant Pathology. Breeding for disease resistance is an important objective of plant breeding. For an effective breeding for resistance, a sound knowledge of plant diseases and their pathogens is essential.

Entomology. Insect pests cause considerable damage to crops. A knowledge of insect pests would be necessary in order to breed insect resistant varieties, and to protect susceptible breeding materials from pest damage.

Bacteriology. Legumes have root nodules containing *Rhizobium*, which fix atmospheric nitrogen. The efficiency of this system depends upon both the host and *Rhizobium* genotypes. Therefore, in legume improvement a knowledge of *Rhizobium* would be helpful. This aspect of legumes is receiving a great deal of attention these days.

Plant Biochemistry. Several types of quality tests are required to determine the quality characteristics of a crop variety. These tests often involve chemical analyses, *e.g.*, protein content, amino acid content etc. A knowledge of biochemistry would be helpful in conducting these tests, and also in developing selection techniques for such characters.

Statistics. For a precise comparison of performances of various varieties, a sound knowledge of statistical methods is a must. The breeder must be well-versed in field plot technique, experimental designs and relevant statistical analyses and tests. The understanding of quantitative inheritance is also based on statistical principles.

In addition to the above, the breeder must be aware of the present market demands, needs of the farmer and the problems of crop production in the concerned area. He should also be able to see into the future to be able to meet the challenges that the farmers may face years later. The breeder has to plan several years ahead because it takes at least 12-15 years to develop and release a new variety.

It is difficult for one person to be specialist in all the above areas. As a result, modern plant breeding is becoming more and more of a team effort. Specialists in genetics, pathology, entomology and agronomy cooperate with the plant breeder in crop improvement. The Indian Council of Agricultural Research (ICAR) has formulated its All India Coordinated Crop Improvement Projects on these lines.

OBJECTIVES OF PLANT BREEDING

Plant breeding aims to improve the characteristics of plants so that they become more desirable agronomically and economically. The specific objectives would vary greatly depending on the crop under consideration. Some of the main objectives of plant breeding may be summarised as follows.

Higher Yields. Most of the breeding programmes aim at higher crop yields. This is achieved by developing more efficient genotypes, *e.g.*, hybrid varieties of maize (*Z. mays*), sorghum (*S. bicolor*), bajra (*P. americanum*), etc.

Improved Quality. The quality of plant produce determines its suitability for various uses. Therefore, quality is an important aspect for plant breeders. The quality characters vary from one crop to another, *e.g.*, grain size, colour, milling and baking quality in wheat (*Triticum aestivum*) ; cooking quality in rice (*Oryza sativa*) ; malting in barley (*Hordeum vulgare*) ; size, colour and flavour of

fruits ; keeping quality of vegetables ; protein content in cereals and legumes ; lysine content in cereals ; methionine and tryptophan contents in pulses etc.

Disease and Insect Resistance. Resistant varieties offer the cheapest and the most convenient method of disease and insect control. In some cases, they offer the only feasible means of control, *e.g.*, rusts in wheat (*T. aestivum*). Resistant varieties not only increase production but also stabilise it.

Change in Maturity Duration. It permits new crop rotations and often extends the crop area. Development of wheat varieties suitable for late planting has permitted rice-wheat rotation. Thus breeding for early maturing crop varieties, or varieties suitable for different dates of planting may be an important objective.

Agronomic Characteristics. Modification of agronomic characteristics, such as plant height, tillering, branching, erect or trailing habit etc., is often desirable. For example, dwarfness in cereals is generally associated with lodging resistance and fertilizer responsiveness.

Photoinsensitivity. Development of photoinsensitive and temperature insensitive wheat and photoinsensitive rice (*O. sativa*) varieties has permitted their cultivation in new areas. Rice is now cultivated in Punjab, while wheat is a major rabi crop in West Bengal.

Synchronous Maturity. It is highly desirable in crops like mung (*Vigna radiata*), where several pickings are necessary.

Nonshattering Characteristics. It would be of great value in a crop like mung.

Determinate Growth. Development of varieties with determinate growth is desirable in crops like mung, pigeon pea (*Cajanus cajan*), cotton (*Gossypium sp.*), etc.

Dormancy. In some crops, seeds germinate even before harvesting if there are rains at the time of maturity, *e.g.*, mung, barley (*H. vulgare*), etc. A period of dormancy in such cases would check the loss due to germination. In some other cases, however, it may be desirable to remove dormancy.

Varieties for New Seasons. Traditionally maize is a kharif crop. But scientists are now able to grow maize (*Z.mays*) as rabi and zaid crops. Similarly, mung is grown as a summer crop in addition to the main kharif crop.

Moisture Stress and Salt Tolerance. Development of varieties for rainfed areas and for saline soils would be helpful in increasing crop production in India. The major proportion (Ca. 70%) of the cropped area in the country is rainfed. The estimates of the salf-affected (saline) soils in the country vary from 7 to 20 million hectares, of which about 2.8 million hectares are alkaline soils. Most of these areas are spread in the states of Utter Pradesh, Haryana and Punjab.

Elimination of Toxic Substances. Some crops have toxic substances which must be eliminated to make them safe for consumption. For example, khesari (*Lathyrus sativus*) seeds have a neurotoxin, β-N-oxalylamine alanine (BOAA) that causes paralysis. Similarly, *Brassica* oil has erucic acid which is harmful to human health. Removal of such toxic substances would increase the nutritional value of these crops.

Winter Hardiness would be desirable in certain situations.

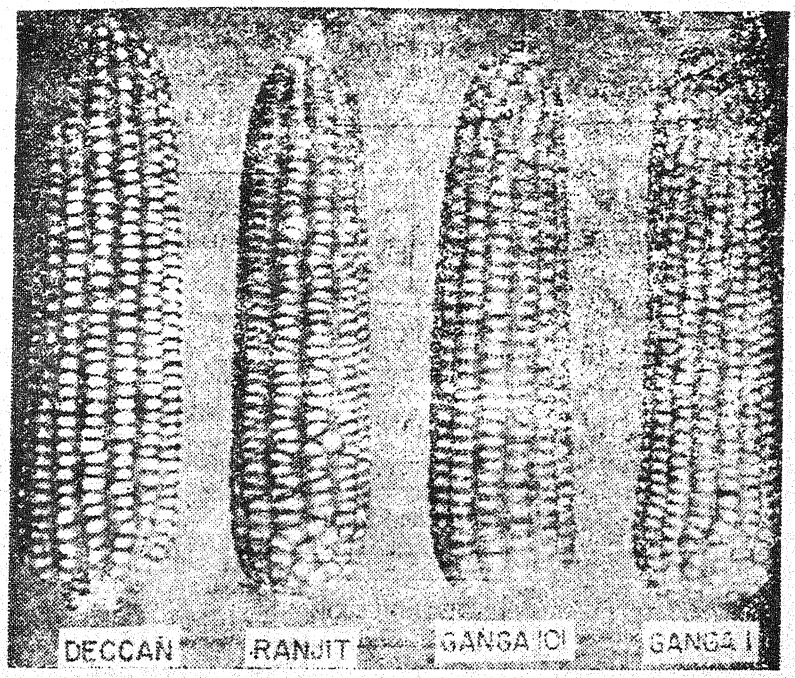


Fig. 1.2. Cobs of improved, high yielding maize hybrids, (Courtesy ICAR).

ACTIVITIES IN PLANT BREEDING

The desired changes in the genotypes of crop species and the consequent benefits to the farmers are brought about by a series of interrelated and largely interdependent activities. These activities are : creation of variation, selection, evaluation, multiplication and

distribution (Fig. 1.3). *Creation of variation* is the prerequisite for

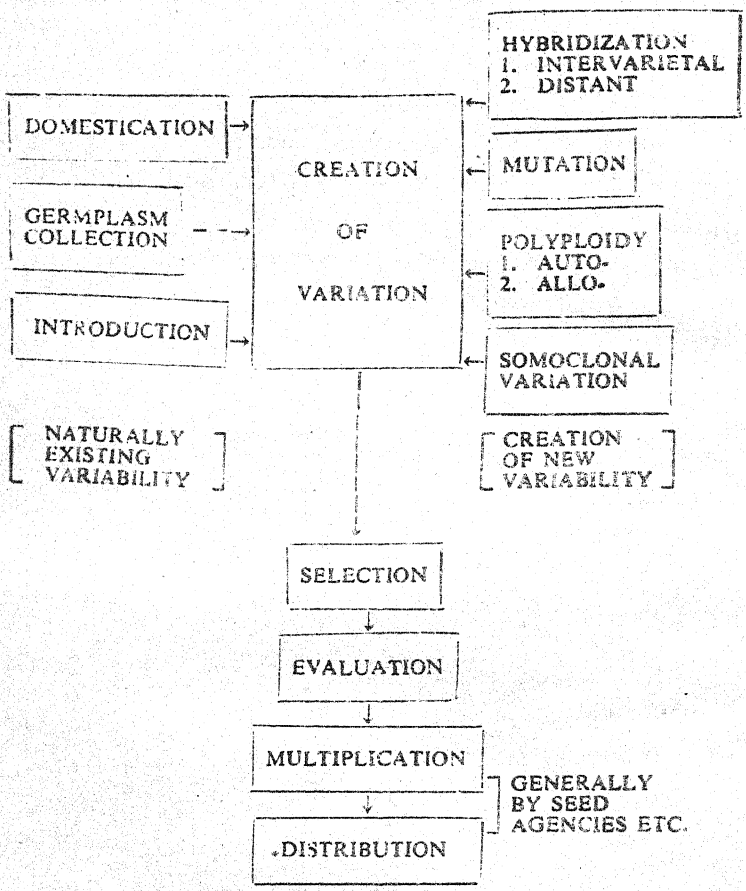


Fig. 1.3. Activities in plant breeding.

any plant breeding programme ; there can be no improvement in the absence of variation. Domestication, germplasm collection, introduction, hybridization (both intervarietal and distant), mutation, polyploidy and somoclonal variation are the chief means for generating variation. *Selection* attempts to isolate the most desirable genotype from the mixture of numerous genotypes in a population. Selection has to be based on phenotype of the plants. Generally, there is highly variable correspondence between the phenotypes and the genotypes of plants. The various selection schemes attempt to overcome this

difficulty and are designed for specific situations, *e.g.*, for self and cross-pollinated species etc. *Evaluation* concerns with the comparison of performance of newly evolved genotypes (strains/selections) with that of the existing varieties. These comparisons are made for two or more years, preferably at several locations, to obtain an estimate of stability in the performance of new strains. The all India Co-ordinated Crop Improvement Projects of ICAR perform this function in an excellent manner. If a newly developed strain is superior to the existing varieties, it is released as a new variety for general cultivation on a commercial scale. The benefits from new variety can be obtained only when the new variety is multiplied and distributed to the farmers. These two functions are performed by various seed agencies in the country.

For an efficient crop improvement programme, the above activities have to be properly coordinated and efficiently geared to maximise the outputs from the programme. A deficiency or slackness in any one of these steps will definitely reduce the efficiency of the programme. This will lead to a wastage of valuable resources, which no nation, particularly a developing nation, can afford. Therefore, a regular and critical review of the progress and the problems has to be made, the deficiencies in the various activities have to be identified and appropriate remedial measures have to be taken to ensure continued progress in breeding programmes.

SOME IMPORTANT ACHIEVEMENTS

The present-day crop plants are very different from the wild species, mostly weeds, from which they originated. This change has been brought about by man through plant breeding. The extent of this change is often so great that it is difficult to realise that the cultivated plants did come from the weedy wild species. This can be appreciated by comparing the cobs of a local variety of maize (*Z. mays*) with those of 'Ganga 101', an improved maize variety (Figs. 1.2, 1.4). Clearly, the present-day crop plants were virtually reconstructed by man from weedy wild plants.

One of the most important developments of modern agriculture has been the production of semidwarf cereal varieties, particularly of wheat and rice. The semidwarf wheat (*T. aestivum*) varieties were developed by N.E. Borlaug and his associates at CIMMYT (International Centre for Wheat and Maize Improvement), Mexico. They used a Japanese variety Norin 10 as the source of dwarfing genes. In 1963, ICAR introduced several dwarf selections from CIMMYT. Kalyan Sona and Sonalika were selected from these materials. For more than one decade, these varieties were the most popular wheat varieties in India. A great majority of the wheat varieties now grown in the country are semidwarf. These semidwarf wheat varieties are lodging resistant, fertilizer responsive and high yielding. They are generally resistant to rusts and other major diseases of

wheat due to incorporation of resistance genes in their genotype. This has greatly increased and stabilised wheat production in the country. These varieties are photoinsensitive and many of them are suitable for late planting. This has enabled cultivation of wheat in nontraditional areas like West Bengal.

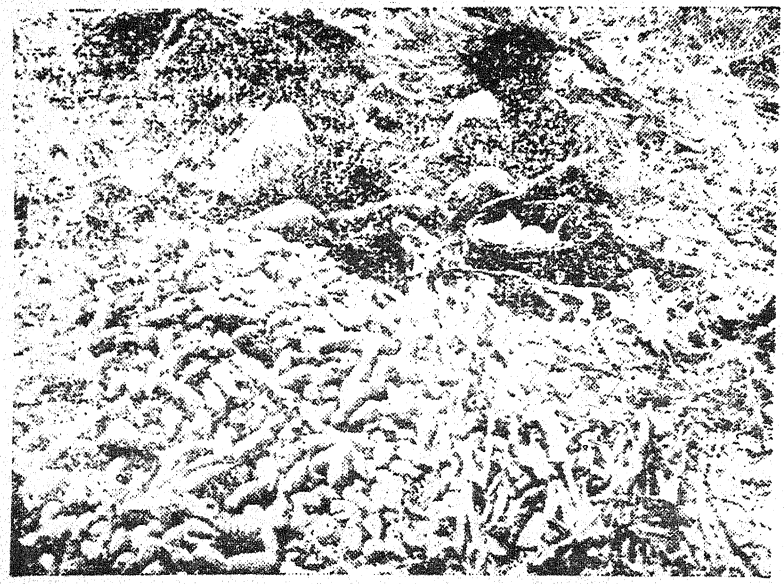


Fig. 1.4. Cobs of a local open-pollinated variety of maize from A.P. (Courtesy, ICAR)

Similarly, the development of semidwarf rice (*O. sativa*) varieties has revolutionised rice cultivation. These varieties were derived from Dee-geo-woo-gen, a dwarf, early-maturing variety of *japonica* rice from Taiwan, Taichung Native I (TN1), developed in Taiwan, and IR 8, developed at IRRI (International Rice Research Institute), Philippines, were introduced in India in 1966. They were extensively grown for few years, but were later replaced by superior semidwarf varieties developed in India, *e.g.*, Jaya, Ratna etc. The semidwarf rice varieties are lodging resistant, fertilizer responsive, high yielding and photoinsensitive. Photoinsensitivity has allowed rice cultivation in nontraditional areas like Punjab. Even in traditional areas, rice-wheat rotation has become possible only due to these varieties.

Another noteworthy achievement is noblisation of sugarcane. The Indian canes were of *Saccharum barberi* origin and were largely grown in North India. They were hardy, but poor in yield and

sugar content. The tropical noble canes of *Sccharum officinarum* origin had thicker stem and higher sugar content. But they performed badly in North India primarily due to low winter temperatures in this region. C.A. Barber, T.S. Venkatraman and others at the Sugarcane Breeding Institute, Coimbatore, transferred the thicker stem, higher sugar content and other desirable characters from the noble canes to the Indian canes. This is commonly referred to as *noblisation of Indian canes*. They also crossed *Saccharum spontaneum* (vern. *kans*), a wild species, to transfer disease resistance and other desirable characteristics to cultivated varieties. Several high yielding varieties with high sugar content and well adapted to local climate have resulted from this breeding programme. At present, sugarcane breeding all over the world is based on the noblisation technique.

The development of hybrid varieties of maize (*Z. mays*), jowar (*S. bicolor*) and bajra (*P. americanum*) deserves a special mention. A programme to develophybrid maize began in India about two decades ago in collaboration with Rockefeller and Ford Foundations. Several hybrid varieties have been released since then. The Ganga series of hybrids, *e.g.*, Ganga Safed 2, and Deccan, are a few examples. Similarly, several hybrids have been released in jowar, *e.g.*, CSH 1, CSH 2, CSH 3, CSH 4, CSH 5, etc., and in bajra, *e.g.*, PHB 10, PHB 14, BJ 104 and BK 560. For certain reasons, hybrid maize varieties could not become popular with the farmers. But in some states like Karnataka, hybrid varieties occupy large areas. More recently, composite varieties are being developed to overcome the difficulties encountered with the hybrid varieties. For example, Manjari, Vikram, Sona, Vijay and Kisan are some of the notable maize composite varieties. Some recently released composites are : CO1, NLD, Renuka, Kanchan, and Diara 3. The composite varieties often yield as much as the hybrid varieties and do not have the drawbacks of the latter. More notably, the farmers need not replace the seed every year in the case of composite varieties.

India has achieved the distinction of commercially exploiting heterosis in cotton. The first hybrid variety of cotton was H 4 (a hybrid from two *G. hirsutum* strains) ; it was developed by the Gujarat Agriculture University (Surat Station) and released for commercial cultivation in 1970. Since then, several other hybrid varieties, *e.g.*, Jk Hy 1, Godavary, Sugema, H 6 and AHH 468 (all within *G. hirsutum*), Varalaxmi, CBS 156, Savitri, Jayalaxmi and H2HC (all *G. hirsutum* × *G. barbadense*), have been released for cultivation. The hybrid varieties are high yielding, and have high ginning outturn and good fiber quality. They are becoming increasingly popular with the farmers ; according to an estimate, they occupy about 70% of the total area under irrigated cotton (this came to about 1.5 million hectares in 1985-86). It is noteworthy that the farmers are more than willing to pay the high cost of the hybrid seed (around Rs 50.00 per kg), which is produced by hand emasculation and hand pollination. Efforts are being made to utilize male sterility for hybrid seed production ; this is expected to reduce the seed cost.

These are only a few examples of the achievements of plant breeding in India. If we consider the achievements in other countries as well, the examples will be far too many to be listed. (1) The improved varieties generally yield 50-80 per cent more than the existing local varieties ; often the yields are much higher. Further, (2) due to resistance to diseases the improved varieties save the cost of plant protection ; they also stabilise crop yields. In addition, (3) they offer numerous other advantages (consider the points listed under *objectives of plant breeding*). Thus plant breeding is of immense economic importance to a nation, especially to that with an agriculture-based economy, like India. This fact is well appreciated by the Government of India. This is evident from the elaborate All India Coordinated Crop Improvement Projects for most of the important crops (*Chapter 28, Table 28.3*).

UNDESIRABLE CONSEQUENCES

In general, improvement in the yield of a crop species is accompanied with a *reduction in variability* among the cultivated varieties of that species. This reduction is produced by two potent factors : *first*, replacement of heterogeneous local varieties by few dominant, more homogeneous improved varieties, and *second*, use of similar/related varieties as parents in breeding programmes. The improved varieties are commonly purelines (self-pollinated species) or hybrids (cross-pollinated species), which are much more homogeneous than the unimproved local and open-pollinated varieties. A few improved varieties become predominant and rapidly replace the local varieties leading to a rapid depletion of genetic variability (*genetic erosion*). This, in turn, limits the prospects of further improvement in the species since variability is the prerequisite for any modification in the characteristics of a crop species. Germplasm collections aim at minimising the detrimental effects of genetic erosion by collecting and preserving the variability in crops and their related species.

Many of the improved varieties have one or more parents (immediate or somewhat removed in the ancestry) in common with each other. For example, many semidwarf varieties of rice have IR 8 or TN 1 as one of their parents ; IR 8 and TN 1 are related to each other by the common parent Dee-geo-woo-gen, the source of their semidwarfing gene. Similarly, almost all the semidwarf wheat varieties have *Rht 1*, *Rht 2* or both these genes for reduced height ; these genes have been derived from a single wheat variety, Norin 10. Thus the improved varieties of a crop species are becoming increasingly similar to each other due to the commonness of one or more parents in their ancestry. This has led to the narrowing down of the genetic base of these varieties ; *genetic base* refers to the genetic variability among the varieties of a crop species. The narrow genetic base has created *genetic vulnerability*, which refers to susceptibility of most of the cultivated varieties of a crop species to a disease, insect pest or some other stress due to the similarity in their genotypes. An example of genetic vulnerability is the outbreak in

epidemic proportions of *Helminthosporium* (now, *Cochliobolus*) leaf blight of maize in the 1970s in U.S.A. This occurred due to the extreme susceptibility of most of the commercial hybrids to the leaf blight ; this susceptibility was produced by the Texas male sterility cytoplasm present in these hybrids. A similar case in India is the susceptibility of early bajra hybrids to downy mildew and ergot ; again this susceptibility was contributed by the male sterile parent of these hybrids, Tift 23A or its dwarf derivative, Tift $23D_2A$. But in this case, the susceptibility was due to some nuclear genes and not due to the male sterile cytoplasm. Genetic vulnerability can be avoided by using diverse and unrelated parents in breeding programmes, and by using unrelated sources of male sterility, semidwarfness etc. Breeders are becoming increasingly aware of this problem and they are making conscious efforts to broaden the genetic base of the cultivated varieties.

Another problem has been generated by the lopsided emphasis on breeding for resistance to the major diseases and insect pests only. This has often resulted in an increased susceptibility to thus far minor diseases. As a result, these diseases have gained in importance and, in some cases, produced severe epidemics. A case in point is the epidemic caused by *Botrytis cinerea* (grey mold) in chickpea during 1980-81 and 1981-82 crop seasons in Punjab, Haryana and parts of U.P. and Bihar. Another example is the severe infection by Karnal bunt (*Tilletia* sp.) on some wheat varieties, *e.g.*, WL 711. This problem is difficult to overcome since resource constraints may never permit a breeder to screen his material against all the diseases and pests of a crop species. We may probably have to, so to say, learn to live with this problem, and tackle it as and when it arises.

Sooner or later, the variability for yield in a crop species is exhausted and no further yield increases are obtained through breeding, *i.e.*, the yield reaches a plateau. Such plateaus were evident in wheat and rice yields before the exploitation of semidwarfing genes, and are being experienced once again. In such cases, new variability has to be introduced in the breeding programmes in order to break the plateau. There is an increasing evidence that wild relatives of cultivated species may provide 'yield genes' for breaking the yield plateaus. In some cases, a novel breeding approach may be useful in raising crop yields beyond the plateau, *e.g.*, hybrid rice.

FUTURE PROSPECTS

The past achievements of plant breeding fully illustrate its future possibilities. The improvements made in the crop plants so far represent only a small portion of the possible improvements. There is considerable scope for further modifying the present-day crop species. It is believed that the genetic makeup of the plants may be modified to a much greater extent than we normally appreciate. Further, breeding of several crop plants, like pulses and oilseeds, has not been so intensive as that of wheat and rice. Much improvement in yields and other characteristics can be made in these crops. Plant breeding,

together with improved crop management practices, is the only answer to the ever increasing demand for foodgrains.

SUMMARY

Plant breeding is a science based on principles of genetics and cytogenetics. It aims at improving the genetic makeup of the crop plants. Improved varieties are developed through plant breeding. Its objectives are to improve yield, quality, disease resistance, drought and frost tolerance and other characteristics of the crops. Plant breeding has been crucial in increasing agricultural production. Some well known achievements are development of semidwarf wheat and rice varieties, noblisation of Indian canes, and production of hybrid and composite varieties of maize, jowar and bajra. Plant breeders should be able to make similar contributions in the future as well.

QUESTIONS

1. Briefly discuss the various factors responsible for low agricultural productivity in India. Explain the contributions of plant breeding in improving the agricultural productivity.
2. List the contributions of the following scientists : (i) Thomas Fairchild, (ii) Joseph Koelreuter, (iii) Andrew Knight, (iv) Le Couteur and Shireff, (v) Vilmorin, (vi) Nilsson-Ehle, (vii) Johannsen, (viii) G.J. Mendel, and (ix) G.H. Shull.
3. Describe the various objectives of plant breeding with the help of suitable examples.
4. Describe in brief how plant breeding has helped in improving the productivity of the following crops in India : (i) Wheat, (ii) Rice, (iii) Maize, and (iv) Sugarcane. Discuss the future prospects of improving crop plants through plant breeding.
5. Briefly describe the history of plant breeding. Discuss the nature of plant breeding, and briefly outline the disciplines with which a plant breeder ought to be familiar for effective crop improvement.

Suggested Further Reading

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CHAPTER 2

Domestication, Plant Introduction and Acclimatisation

DOMESTICATION

Domestication is the process of bringing wild species under human management. The present-day cultivated plants have been derived from wild weedy species. The first step in the development of cultivated plants was their domestication. Most of the crops were domesticated by the prehistoric man. Knowingly or unknowingly he must have selected for the characteristics that made the plants more suited to his needs. Under domestication, the crop species have changed considerably as compared to the wild species from which they originated. The change is often so great that they are classified as distinct species. As a result, in many cases, the parental wild species of the cultivated plants are not definitely known. This great difference between cultivated plants and their wild relatives was brought about by selection by man as well as nature. The domesticated species were selected for characteristics entirely different from those for which the wild species were selected in nature. Therefore, the two groups of plants developed in two different, often opposite, directions.

Domestication of wild species is still being done and is likely to continue for a long time in the future. This is because the human needs are likely to change with time. Consequently, the wild species of little importance today may assume great significance tomorrow. This is particularly true for microorganisms producing antibiotics, involved in nitrogen fixation, and producing other compounds of industrial or medical interest ; forest trees producing timber and other commercial products ; medicinal plants ; and plants fulfilling specific needs. A notable case of recent dometication is that of several members of Euphorbiaceae producing latex. The latex of these plants may be commercially used for extraction of petroleum products, including petrol and diesel. A large scale cultivation of these plants is being done in U.S.A. and Japan. The Department of Science and Technology, Government of India has initiated a

project for cultivation of jojoba (*Simmondsia* sp.) in arid zones of Rajasthan, Gujarat, Maharashtra and Uttar Pradesh. Seeds of jojoba contain oil which is comparable to sperm whale oil and is highly suitable as an industrial lubricant. The plants producing latex are gopher plant (*Hevea* sp.), milkweed (*Euphorbia lathyrus*) etc. They are hardy desert plants and their latex compares favourably with petroleum crude, and is being used for extraction of petroleum products. As a result, fields of these plants are often called *living oil fields*.

Natural and Artificial Selection under Demestication

The precise sequence of events in evolution of crop plants under domestication is not known. But it may be generalised that in the early stages a considerable variability existed in the domesticated species. New variability arose from hybridization followed by recombination, and from mutation. The extent and manner of selection exerted by man on the domesticated species is not known. *Selection may be described as a phenomenon of some genotypes from a population leaving behind more progeny than others.* The genotypes that produce more progeny are selected for and the others are selected against. In nature, there is a continuous selection by natural forces, *e.g.*, temperature, soil, weather, pests, diseases, etc. As a result, *the genotypes more suited to a given environment leave behind more progeny than the less adapted ones. This process is known as natural selection.* Thus natural selection is one of degree and it rarely stops a genotype from producing some progeny. The selection by man (*artificial selection*), on the other hand, often permits only the selected plants to reproduce ; the progeny from the remaining plants are generally discarded. Thus natural selection retains considerable variability in the species, while artificial selection progressively reduces this variability.

There is no doubt, however, that man exerted considerable selection on domesticated plant species. It may be expected that he would have selected those plant types that best suited his needs. It is likely that he selected for larger fruit and grain sizes. It may be expected that his methods of selection were primitive in that they were not based on scientific principles. His sole criterion of selection would have been the phenotype of plants. But there could be no doubt that this, combined with the natural selection under domestication, was highly effective. Artificial and natural selections have led to several distinct changes in the characteristics of domesticated species.

Changes in Plant Species under Domestication

Almost all the characteristics of plant species have been affected under domestication. The characters that show more distinct changes are those that have been objects of selection and are still plant breeding objectives in many cultivated species. Some of the important changes that have occurred under domestication are briefly listed below.

1. Elimination of or reduction in shattering of pods, spikes etc. This change has taken place in most of the cultivated species.
2. Elimination of dormancy has taken place in several crop species. Lack of dormancy has become a problem in crops like barely (*H. vulgare*), wheat, (*T. aestivum*), mung (*Vigna radiata*) etc.
3. Decrease in toxins or other undesirable substances. The bitter principle of cucurbitaceous plants is an example.
4. Plant type has been extensively modified. The cultivated plants show altered tillering, branching, leaf characters, etc.
5. In several crop species, there has been a decrease in plant height, *e.g.*, cereals, millets. This is often associated with a change from indeterminate to determinate habit.
6. In some species, on the other hand, there has been an increase in plant height under domestication, *e.g.*, jute (*Corchorus* sp.), sugarcane (*S. officinarum*), forage grasses etc.
7. Life cycle has become shorter in case of some plant species. This is particularly so in case of crops like cotton (*Gossypium* sp.), arhar (*Cajanus cajan*), etc.
8. Most of the crop plants show an increase in size of grains or fruits.
9. Increase in the economic yields is the most noticeable as well as desirable change under domestication. This is self-evident in every crop species.
10. In many crop species, asexual reproduction has been promoted under domestication, *e g.*, sugarcane, potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*), etc.
11. There has been a preference for polyploidy under domestication. Many of the domesticated plant species are polyploids, *e.g.*, potato, wheat, sweet potato, tobacco *Nicotiana* sp., etc., while diploid plants are present in nature. Thus domestication seems to have favoured polyploidy in these crop species.
12. In many species, there has been a shift in the sex form of the species. In many dioecious fruit trees, bisexual forms have developed under domestication. Self-incompatibility has also been eliminated in many crop species.
13. Variability within a variety has drastically decreased under domestication. The extreme case is that of pureline varieties which are completely homogeneous genotypically.

PATTERNS OF EVOLUTION IN CROP PLANTS

It is apparent that selection by nature and man has been responsible for evolution of crop plants. However, selection is

effective in altering a species only when variability exists in the populations of that species. There are three major ways in which variability has arisen in the crop species. The patterns of evolution of various crops may be broadly classified according to the mode of origin of variation crucial for evolution of that species.

Mendelian Variation. Many crops have evolved through variation generated by gene mutations, and by hybridization between different genotypes within the same species followed by recombination. Ultimately, all the variability in any species originates from gene mutations. Most of the gene mutations are harmful and are eventually eliminated. But some mutations are beneficial and are retained in the population. The mutations may be grouped into two categories : macromutation and micromutation. *A macromutation produces a large and distinct morphological effect, and often affects several characters of the plant.* A single macromutation has led to the differentiation of modern maize (*Z. mays*) plant from the grassy pod corn. This mutation has affected the position of male and female inflorescences, the habit of the plant and several other characters. Similarly, cabbage (*Brassica oleracea*), cauliflower (*B. oleracea*), broccoli (*B. oleracea*), and Brussel's sprouts (*B. oleracea*) have originated from a common wild species and they differ from each other with respect to a few major genes.

The greater part of variation, however, has resulted from *mutations with small and less drastic effects, i.e., micromutations.* Since micromutations have only small effects, they tend to be accumulated in a population. Natural selection would accumulate and select for more favourable gene combinations. Man would have selected from the populations desirable plant types leading to differentiation of the domesticated species from the wild ones. Several important crops have evolved through Mendelian variation, *e.g.*, barely, rice beans (*Phaseolus* sp.), peas (*Pisum sativum*), tomatoes (*Lycopersicon esculentum*), linseed (*Linum usitatissimum*), jowar, bajra and many other crops.

Interspecific Hybridization. *Interspecific hybridization refers to crossing of two different species of plants.* The resulting F_1 is generally more vigorous than the parents. But segregation in F_2 and later generations produces a vast range of genotypes. This is because the parental species are likely to differ from each other for a large number of genes. Most of the recombinants in the segregating generations are likely to be weak and undesirable. Often interspecific hybrids are highly sterile and do not set seeds. There is little evidence to suggest that interspecific hybridization contributed to any great extent in evolution of crop species.

But in some cases, the interspecific hybrids may have repeatedly backcrossed to one of the parental species. Thus most of the genotype of that parental species would be recovered alongwith few or several genes from the other parental species. This process is known as *introgressive hybridization and leads to the transfer of some genes*

from one species into another. The modern maize (*Z. mays*) is postulated to have developed through introgressive hybridization between the primitive maize and a wild grass, *Tripsacum.* It is supposed that some genes from *Tripsacum* were transferred to the primitive maize which resulted in the modern maize.

Interspecific hybrization has led to the development of several strawberry varieties. The F_1 from a cross between two species of strawberries, *Fragaria virginiana* and *Fragaria chiloensis,* was back-crossed to the two parent species to produce many varieties of commercial value. In certain fruit trees, such as pears, plums, cherries and grapes, and ornamentals, *e.g.*, irises (*Iris* sp.), roses (*Rosa* sp.), lilies (*Lillium* sp.) etc., vegetative propagation is commonly used. In such species, many varieties are interspecific hybrids (F_1).

Polyploidy. Generally, autopolyploidy leads to increased vigour, larger flowers and fruits etc. over the diploid forms. Many varieties of ornamental plants are autopolyploids. The commercial banana (*Musa paradisiaca*) is an autotriploid (3*x*) ; it has larger and seedless fruits in comparison to the diploid banana. Triploid varieties are known in apples (*Pyrus malus*), watermelons (*Citrullus vulgaris*), sugar beets (*Beta vulgaris*) and some other crops. The commonly grown potato (*Solanum tuberosum*) may be regarded as an autotetraploid, although interspecific hybridization may also be involved. *S. tuberosum* has 3*x*, 2*x* and 4*x* types. Some of the 2*x* progeny obtained from the 4*x* potato are fully fertile and as vigorous as the 4*x* types, indicating that it is largely an autotetraploid. Other autopolyploid crop species are sweet potato (6*x*), oat (*Avena abyssinica,* 4*x*) and alfalfa (*Medicago sativa,* 4*x*). Thus autopolyploidy has played a limited role in crop evolution.

Allopolyploidy, in contrast, has been considerably more important in crop evolution. Allopolyploidy results from chromosome doubling of interspecific F_1 hybrids. About 50 per cent of the crop plants are allopolyploids. Some of the important allopolyploid crop plants are wheat, tobacco, cotton, sugarcane, oats (*Avena* sp.), rai (*Brassica juncea*), rapeseed (*Brassica napus*) etc. Origins of wheat, tobacco, cotton, and oats have been extensively investigated. Common bread wheat (*Triticum aestivum*) is an allohexaploid, while cotton (*Gossypium hirsutum* and *G. barbadense*) and tobacco (*Nicotiana tabacum* and *N. rustica*) are allotetraploids. There is evidence to suggest that *N. tabacum* originated from chromosome doubling of the F_1 hybrid from *N. sylvestris* × *N. tomentosa. Triticale* is a man made allopolyploid developed by chromosome doubling of the F_1 between rye (*Secale cereale*) and tetraploid or hexaploid wheats. *Triticale* has shown much promise particularly in areas of moisture or temperature stress.

CENTRES OF ORIGIN

There is considerable evidence that the cultivated plants were not distributed uniformly throughout the world. Even today,

certain areas show far greater diversity than others in the forms of certain cultivated crops and their wild relatives. N.I. Vavilov proposed that *crop plants evolved from wild species in the areas showing great diversity and termed them as primary centres of origin.* Later, crops moved to other areas primarily due to the activities of man. These areas generally lack the richness in variation found in the primary centres of origin. *But in some areas, certain crop species show considerable diversity of forms although they did not originate there. Such areas are known as secondary centres of origin of these species.*

The concept of centres of origin was given by Vavilov based on his studies of a vast collection of plants at the Institute of Plant Industry, Leningrad. He was director of this institute from 1916 till 1936. He also postulated the *Law of Homologous Series in Variation. This law states that characters found in one species also occur in other related species.* Thus diploid (2*x*), tetraploid (4*x*) and hexaploid (6*x*) wheats show a series of identical contrasting characters. Similarly, genus *Secale* duplicates the variation found in *Triticum.* Thus a character absent in a species, but found in a related species, is likely to be found in the collections of that species from the centre of its origin.

Eight main centres of origin are recognised as proposed by Vavilov : China, Hindustan, Central Asia, Asia Minor, Mediterranean, Abyssinia, Central and South America.

The China Centre of Origin. This centre consists of the mountaineous regions of central and western China and the neighbouring lowlands. It is the largest and the oldest independent centre of origin. The crops that originated in this area (*primary centre of origin*) are : soybeans (*Glycine max*), radish (*Raphanus sativus*), *Colocasia antiquorum* (bunda), *Panicum miliaceum* (proso millet), and some other, species of millets, buck wheat (*Fagopyrum escluentum*), *Papaver somniferum* (opium poppy), several species of *Brassica* and *Allium*, *Solanum melongena* (brinjal), some species of *Cucumis* and *Cucurbita*, pears (*Pyrus communis*), peaches (*Prunus persica*), apricots (*Prunus armeniaca*), plums (*Prunus divaricata*), orange (*Citrus nobilis*), Chinese tea (*Camellia sinensis*) and naked oats (*Aveda nuda*).

In addition, it is *secondary centre of origin* for several crop plants, *e.g., Zea mays* (maize), *Phaseolus vulgaris* (rajma), cowpea (*Vigna anguiculata*), turnip (*Brassica rapa*) and sesame or til (*Sesamum indicum*).

The Hindustan Centre of Origin. This centre includes Burma, Assam, Malaya Archipelago, Java, Borneo, Sumatra and Philippines, but excludes North-west India, Punjab and North-western Frontier Provinces. It is the *primary centre of origin* of rice (*Oryza sativa*), arhar (*Cajanus cajan*), gram or chickpea (*Cicer arietinum*), cowpea (*V. anguiculata*), mung (*V. radiata*), brinjal (*S. melongena*), *Cucumis sativus* (cucumber), *Lactuca indica* (Indian lettuce), certain species

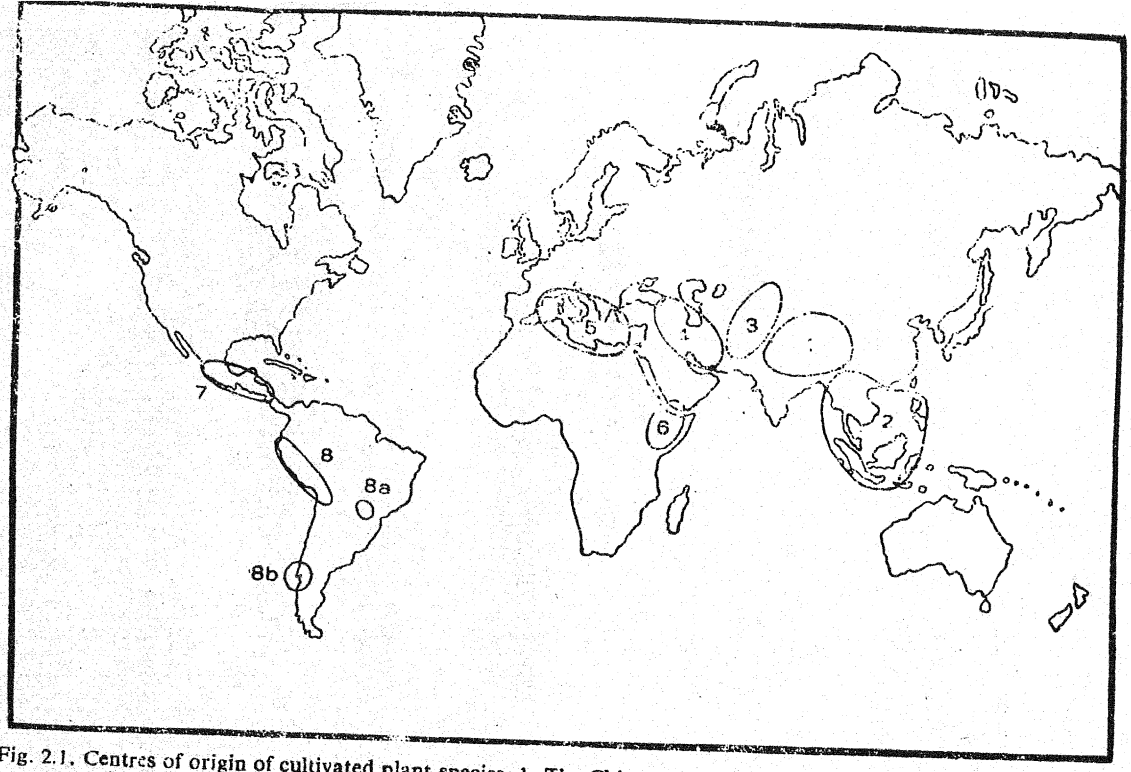


Fig. 2.1. Centres of origin of cultivated plant species. 1. The China Centre, 2. The Hindustan Centre, 3. The Central Asia Centre, 4. The Asia Minor Centre, 5. The Mediterranean Centre, 6. The Abyssinian Centre, 7. The Central American Centre, and 8. The South American Centre (8. Peru, 8a. Brazil—Paraguay, and 8b. Chile).

of *Dioscorea* (yams), *Raphanus indicus* (Indian radish), *Saccharum officinarum* (noble canes), several species of cotton (particularly, *Gossypium arboreum*), hemp (*Cannabis indica*), black pepper (*Piper nigrum*), indigo (*Indigofera* sp.), mango (*Mangifera indica*), orange, sour lime and some other *Citrus* species, coconut (*Cocos nucifera*), banana (*Musa sapientum*) and turmeric (*Curcuma domestica*).

The Central Asia Centre of Origin. It includes North-west India (Punjab, The North-west Frontier Provinces and Kashmir), all of Afghanistan, the Soviet Republics of Tadjikistan and Uzbekistan and Tian-Shan. It is also known as the *Afghanistan Centre of Origin.* The crops that originated in this centre (*primary centre of origin*) are : wheat (*Triticum aestivum*), club wheat (*Triticum compactum*), pea (*Pisum sativum*), broad bean (*Vicia faba*), mung (*Vigna radiata*), linseed (*Linum usitatissimum*), sesame (*S. indicum*), safflower (*Carthamus tinctorius*), hemp, cotton (*Gossypium herbaceum*), radish (*R. sativus*), musk melon (*Curcurbita moschata*), carrot (*Daucus carota*), onion (*Allium cepa*), garlic (*Allium sativum*), spinach (*Spinacea oleracea*), pistachio nut (*Pistacia vera*), apricot (*Prunus armeniaca*), pear (*Pyrus communis*), almond (*Prunus amygdalus*), grape (*Vitis vinifera*) and apple (*P. malus*). It is *secondary centre of origin* of rye (*Secale cereale*).

The Asia Minor Centre of Origin. This is also known as the *Near East or the Persian Centre of Origin.* It includes the interior of Asia Minor, the whole of Transcaucasia, Iran and Highlands of Turkmenistan. The crop species that originated in this region (*primary centre of origin*) include nine species of *Triticum*, rye (*S. cereale*), alfalfa (*Medicago sativa*), Persian clover (*Trifolium resupinatum*). carrot (*Daucus carota*), cabbage (*Brassica oleracea*), oat (*Avena sativa*), species of *Allium*, lettuce (*Lactuca sativa*), fig (*Ficus carica*), pomegranate (*Punica granatum*), apple, several species of *Pyrus*, *Prunus*, grape (*V. vinifera*), almonds (*P. amygdalus*), chestnuts (*Castanea* sp.) and pistachio nut (*P. vera*). It is the *secondary centre of origin* of rape (*Brassica campestris*), black mustard (*Brassica nigra*), leaf mustard (*Brassica japonica*), turnip (*B. rapa*) and apricot (*P. armeniaca*).

The Mediterranean Centre of Origin. Many valuable cereals and legumes originated in this area. The species that originated in this centre are: durum wheats (*Triticum durum*), emmer wheats (*Triticum, dicoccum*), and other *Triticum* species, several species of *Avena*, barley (*Hordeum valgare*), lentil (*Lens esculenta*), several species of *Lathyrus*, pea (*P. sativum*), broad bean (*V. faba*), lupins (*Lupinus* sp.), chickpea (*C. arietinum*), clovers (*Trifolium* sp.), vetch (*Vicia sativa*), several species of *Brassica*, such as rape and black mustard, cabbage and turnip, onion (*A. cepa*), garlic (*A. sativum*), beets (*Beta vulgaris*), lettuce (*Lactuca sativa*), artichoke, asparagus (*Asparagus officinalis*), lavender, peppermint (*Mentha* sp.) and sage.

The Abyssinian Centre of Origin. It includes Ethiopia and hill country of Eritrea. It is the centre of origin for *H. vulgare* (barley),

Triticum durum, *Triticum turgidum*, *Triticum dicoccum*, jowar (*Sorghum bicolor*), bajra (*Pennisetum americanum*), gram, lentil (*L. esculenta*), sem (*Dolichos lablab*), pea, khesari (*Lathyrus sativus*), linseed (*L. usitatissimum*), safflower (*C. tinctorius*), sesame, castor (*Ricinus communis*), coffee (*Coffea arabica*), onion and okra (*Abelmoschos esculentus*). It is *secondary centre of origin* for broad bean (*Vicia faba*).

Central American Centre of Origin. This includes the region of South Mexico and Central America. It is also referred to as the *Mexican Centre of Origin*. The plants that originated here are maize (*Zea mays*), rajma (*P. vulgaris*), lima bean (*Phaseolus lunatus*), melons, pumpkin (*Cucurbita melanosperma*), sweet potato (*Ipomoea batatas*), arrowroot (*Canna edulis*), chillies (*Capsicum annuum*), cotton *G. hirutum* and *G. purpureascens*), papaya (*Carica papaya*), guava (*Psidium guayava*) and avacado (*Persea americana*).

The South American Centre of Origin. This centre includes the high mountainous regions of Peru, Bolivia, Ecuador, Colombia, parts of Chile and Brazil, and whole of Paraguay. The crops that originated in this centre (*primary centre of origin*) are many species of potatoes, maize, lima bean, peanut (*Arachis hypogaea*). pineapple (*Ananas comosa*), pumpkin (*Cucurbita maxima*), Egyptian cotton (*Gossypium barbadense*), tomatoes, guava, tobacco (*Nicotiana tabacum* and other species), quinine tree (*Cinchona calisaya*), cassava (*Manihot utilissima*), and rubber (*Hevea* sp.).

Later, in 1935, Vavilov divided the Hindustan Centre of Origin into two centres, *viz.*, *Indo-Burma* and *Siam-Malaya—Java Centres of Origin*. The South American Centre was divided into three centres, namely, *Peru*, *Chile* and *Brazil-Paraguay Centres of Origin*. Thus the eight main centres were grouped into 11 centres of origin. At the same time he introduced a new centre of origin, the *U.S.A. Centre of Origin*. Two plant species, sunflower (*Helianthus annuus*) and Jerusalem artichoke (*Helianthus tuberosus*), originated in the U.S.A. centre of origin.

The concept that centres of diversity represent centres of origin has been seriously questioned. Plants of a species growing in different environments are likely to be different, *i.e.*, diverse. Thus a species is likely to show a greater variation in a region with varied climatic and other ecological conditions. Areas with mountains and valleys show considerable variation in environment. Therefore, plant species would show a greater variation in such areas. The centres of origin are situated in such mountain-valley areas. Further the centres of diversity of many species have shifted with time. This shift in diversity was brought about by a shift in the area of the greatest cultivation and due to introduction of the species in an area with a greater ecological diversity. These processes have given rise to the *secondary centres of diversity*. Consequently, several species have two or more centres of diversity and it is often difficult to determine which one of them is the real centre of origin.

Thus the centres of origin may be more appropriately called the centres of diversity. These centres may not be the centres of orgin of the species concerned, but they are the areas of the maximum diversity of these species. This serves as an extremely useful guide to plant explorers as to where to search for variation in a given species. Within the large centres of diversity, small areas may exhibit much greater diversity than the centre as a whole. These areas are known as *microcentres.* The crop evolution appears to proceed at a more rapid rate in the microcentres. The microcentres are important for plant collection as well as for experimentally studying the evolution of plants.

PLANT INTRODUCTION

Plant introduction consists of taking a genotype or a group of genotypes of plants into new environments where they were not being grown before. Thus introduction may involve new varieties of a crop already grown in the area, a wild relative of the crop species or a totally new crop species for the area. Often the materials are introduced from other countries or continents. But movement of crop varieties from one environment to another within a country is also introduction. Some examples of within the country introduction are the popularisation of grape cultivation in Haryana, of wheat in West Bengal and of rice in Punjab. Introduction may be classified into two categories, primary and secondary.

Primary Introduction. *When the introduced variety is well suited to the new environment, it is released for commercial cultivation without any alteration in the original genotype. This constitutes primary introduction.* Primary introduction is less common, particularly in countries having well organised crop improvement programmes. Introduction of semidwarf wheat (*Triticum aestivum*) varieties Sonora 64 and Lerma Rojo, and of semidwarf rice (*O. sativa*) varieties Taichung Native 1, IR 8 and IR 28, IR 36 are some examples of primary introduction in this country.

Secondary Introduction. *The introduced variety may be subjected to selection to isolate a superior variety. Alternatively, it may be hybridized with local varieties to transfer one or few characters from this variety to the local varieties. These processes are known as secondary introduction.* Secondary introduction is much more common than primary introduction. Examples of secondary introduction are : Kalyan Sona and Sonalika wheat varieties selected from materials introduced from CIMMYT, Mexico ; semidwarf wheat and rice varieties developed through hybridization with introduced varieties, etc.

HISTORY OF PLANT INTRODUCTION

Crop plants have travelled into many new areas from their centres of origin. This movement of plants occurred with the move-

ment of man. Most of these introductions occurred very early in the history. For example, mung, mustard (*Brassica juncea*), pear, apple and walnut (*Juglans regia*) were introducted from the Central Asian Centre of Origin into various parts of India in the dim past. Similary, sesame, jowar, arhar (*C. cajan*), Asian cotton (*Gossypium herbaceum*) and finger millet (*Eleusine coracana*) originated in Africa and travelled to India in the prehistoric period. It is, therefore, clear that the plant wealth of various nations is, to a large extent, the result of plant introductions.

For several centuries A.D., the agencies of plant introduction were invaders, settlers, traders, travellers, explorers, pilgrims and naturalists. The plant introductions were made either knowingly or unknowingly. Muslim invaders introduced in India cherries and grapes from Afghanistan by 1300 A.D. In the 16th century A.D., Portuguese introduced maize, groundunt, chillies, potato, sweet potato, guava, pineapple, papaya, cashewnut (*Anacardium occidentale*) and tobacco. East India Company brought tea (*Camellia sinensis*), litchi and loquat from China ; cabbage, cauliflower and other vegetables from the Mediterranean; annatto (*Bixa arellana*—a source of edible dye) and mahogany timber tree from the West Indies in the last quarter of 18th century.

During 19th century, a number of Botanic Gardens played an important role in plant introduction. The Calcutta Botanic Gardens was established in 1781. The Kew Botanic Gardens, England, arranged introduction of quinine and rubber trees from South America into India. During and after the last part of 19th century, various agricultural and horticultural research stations were established in the country. These stations introduced horticultural and agricultural plants independent of each other. There was no coordination among these agencies regarding their introduction activities.

PLANT INTRODUCTION AGENCIES IN INDIA

A centralised plant introduction agency was initiated in 1946 at the Indian Agricultural Research Institute (IARI), New Delhi. The agency began as a plant introduction scheme in the Division of Botany and was funded by ICAR. In 1956, during the second five year plan, the scheme was expanded as the Plant Introduction and Exploration Organisation. Subsequently in 1961, it was made an independent division in IARI, the Division of Plant Introduction. The division was reorganised as *National Bureau of Plant Genetic Resources* (*NBPGR*) in 1976. However, the nature of activities and the functions of the bureau have remained the same ; but the scope and scale of its activities have increased considerably. The bureau is responsible for introduction and maintenance of the germplasm of agricultural and horticultural plants.

In addition to the National Bureau of Plant Genetic Resources, there are some other agencies for plant introduction. *Forest*

Research Institute, Dehradun, has a plant introduction organisation which looks after introduction, maintenance and testing of germplasm of forest trees. *The Botanical Survey of India* was established in 1890 ; it was responsible for introduction, testing and maintenance of plant materials of botanical and medicinal interest. But at present, introduction and improvement of medicinal plants is being looked after by the NBPGR. *The Central Research Institutes* for various crops, *e.g.*, tea, coffee, sugarcane, potato, tobacco, rice etc., introduce, test and maintain plant materials of their interest. *But their activities are coordinated by the NBPGR, which has the ultimate responsibility for introduction activities.* Plant materials may also be introduced by individual scientists, universities and other research organisations. *But all the introductions in India must be routed through the NBPGR, New Delhi.*

The National Bureau of Plant Genetic Resources. The bureau has its headquarters at IARI, New Delhi. It has four substations for testing of introduced plant materials. These substations represent the various climatic zones of India. The four substations of NBPGR are listed below :

1. **Simla.** It is situated in Himachal Pradesh and represents the temperate zone ; approximately 2,300 m above sea level.
2. **Jodhpur, Rajasthan.** It represents the arid zone.
3. **Kanya Kumari, Tamil Nadu.** It represents the tropical zone.
4. **Akola, Maharashtra.** It represents the mixed climatic zone. It was recently shifted from Amravati.

In addition, a new substation has recently been established at Shillong for collection of germplasm from North-east India. This part of the country has a large genetic variability for several crop species, *e.g.*, rice, citrus, maize etc.

The bureau functions as the central agency for export and introduction of germplasms of economic importance. The bureau is assisted in its activities by the various Central Research Institutes of ICAR. The activities of the bureau are summarised below.

1. It introduces the required germplasm from its counterparts or other agencies in other countries.
2. It arranges explorations inside and outside the country to collect valuable germplasm.
3. It is responsible for the inspection and quarantine of all introduced plant materials.
4. Testing, multiplication and maintenance of germplasm obtained through various sources. This may be done by the bureau itself at one of its substations or by one of the concerned Central Institutes of ICAR.

5. To supply, on request, germplasm to various scientists or institutions. The germplasm may be supplied ex-stock or may be procured from outside in case it is not available in the country.

6. Maintenance of records of plant name, variety name, propagating material, special characteristics, source, date and other relevant information about the materials received.

7. To supply germplasm to its counterparts or other agencies in other countries.

8. To publish its exchange and collection lists. Introduction News Letter containing such lists is be ng published by Food and Agriculture Organisation (FAO) since 1957 at irregular intervals. NBPGR has also published some lists, and is in the process of publishing some other catalogues.

9. To set up natural gene sanctuaries of plants where genetic resources are endangered.

10. Improvement of certain plants like medicinal and aromatic plants.

PROCEDURE OF PLANT INTRODUCTION

Plant introduction is one of the oldest and very effective methods of plant breeding. The chief function of plant introduction is to make available variation to be utilized in breeding programmes. Introduction consists of the following steps : procurement, quarantine, cataloguing, evaluation, multiplication and distribution.

Procurement of Germplasm. The scientists in this country submit their requirements to the NBPGR. If the bureau is unable to meet the requests from its own stocks or from known sources in the country, it attempts to obtain them from its counterparts in other countries, nurseries, Botanic Gardens or from other agencies. Generally, the materials are obtained through correspondence as gifts, in exchange of germplasm, in consideration of past gifts from the bureau or in anticipation of future gifts. Sometimes, the germplasm has to be purchased. The bureau participates in the activities of the International Board for Plant Genetic Resources (IBPGR). IBPGR aims at free exchange of plant genetic resources, and is often helpful in arranging the supply of needed germplasm. But sometimes it may be necessary to organise explorations to collect the required germplasm.

The plant part to be introduced depends upon the crop species. It may be seeds, rubers, runners, suckers, stolons, blubs, rooted cuttings, buds or sometimes seedlings. *The part of plant used in the propagation of a species is known as propagule.* The nature of propagules varies from species to species. Seeds generally have longer viability than other propagules and are packed and trans-

ported more easily. Other propagules may require special packing techniques. For example, FAO packs 4-5 grass tillers inside polythene bags, and the roots of the tillers are surrounded by moist cotton wool. Techniques are also available for packing other short-life plant propagules. Seeds are generally obtained by surface mail often packed and sealed in think paper envelopes. Other propagules and seeds of such species as mango or *Citrus* are shipped by air mail, preferably through commercial airline. Such materials are personally collected by a representative of the bureau immediately after it receives information of its arrival from the post office or the airline office

Quarantine. *Quarantine means to keep materials in isolation to prevent spreacing of diseases, etc.* All the introduced materials are thoroughly inspected for contamination with weeds, diseases and insect pests. Materials that are suspected to be contaminated are fumigated or are given other treatments to get rid of contamination. If necessary, the materials are grown in isolation for observation of diseases, insect pests and weeds. This entire process is known as *quarantine* and the rules proscribing them are known as *quarantine rules.*

There are several instances where weeds, diseases and pests have been introduced alongwith plant materials (see later). This only emphasizes the necessity for a centralised agency for plant introduction to ensure effective quarantine of the introduced materials. *All the materials being introduced must be covered by an authentic phytosanitory certificate from the source country, i.e., they must be declared free from diseases, weeds and insect pests.* Plant materials being introduced or exported must conform to certain quarantine regulations. The quarantine regulations cover plant propagules, soils, packing materials of plant origin and other suspected objects, Introductions not conforming to quarantine rules, or suspected to be contaminated are likely to be destroyed by NBPGR or would be returned to the source country.

The quarantine control is exercised by the NBPGR at prescribed ports of entry, *e.g.*, Delhi, Bombay, Calcutta, Madras etc. The plant materials accompanied by phytosanitory certificates are thoroughly examined. Materials contaminated by diseases or pests are destroyed or returned to the sender. The materials conforming to the quarantine laws are fumigated against diseases and pests. Sometimes the introduced materials are grown in isolated nurseries or green houses for a critical observation on diseases, pests and weeds. At IARI, units set up in the Divisions of Mycology and Plant Pathology, and Entomology keep the Bureau's nurseries under observation. They assist in evaluation of diseases and pests. This is very important to prevent entry of diseases and pests and weeds because they may not only destroy the introduced plants but also the existing crop species

Restrictions on Export and Introduction of Plant Materials. Introduction into India is restricted in case of certain plant species. Cotton, berseem, linseed and sugarcane seeds cannot be obtained through letter post. Introduction of the following is prohibited : cocoa and other *Theobromo* species (plant and seed) from Africa, Ceylon and West Indies ; sugarcane from Australia, Fiji, New Guinea, Philippines and West Indies ; rubber (plant and seed) from America and West Indies ; and sunflower from Argentina and Peru. Entry of unginned cotton and of Mexican jumping beans is strictly prohibited.

In addition, export of certain plant species is either restricted or prohibited. Almost all countries restrict exchange of germplasm of one or the other plant species. India restricts, more often prohibits, the exports of propagules of tea, jute and black pepper.

Cataloguing. When an introduction is received, it is given an entry number, and information regarding the name of the species and variety, place of origin, adaptation and characteristics are recorded. The plant materials are classified into three groups.

1. Exotic collections are given the prefix 'EC',
2. Indigenous collections are designated as 'IC', and
3. Indigenous wild collections are marked as 'IW'.

Evaluation. To assess the potential of new introductions, their performance is evaluated at different substations of the Bureau. In case of those crops for which Central Research Institutes are functioning, *e.g.*, rice, sugarcane, potato, tobacco etc., the introduced materials are evaluated and maintained by these institutes. The resistance to diseases and pests is evaluated under environments favouring heavy attacks by the diseases and pests.

Acclimatisation. Generally, the introduced varieties perform poorly because they are often not adapted to new environment. Sometimes, the performance of a variety in the new environment improves with the number of generations grown there. *The process that leads to the adaptation of a variety to a new environment is known as acclimatisation.* Acclimatisation is brought about by a faster multiplication of those genotypes (present in the original population) that are better adapted to the new environment. Thus acclimatisation is essentially natural selection. *Variability must be present in the original population for acclimatisation to occur.* Land varieties are likely to get acclimatised, while a purline is not likely to do so.

The extent of acclimatisation is determined by (1) the mode of pollination, (2) the range of genetic variability in the original population, and (3) the duration of life-cycle of the crop. Cross-pollination leads to far more gene recombinations than self-pollination. As a result, cross-pollination is much more helpful in accli-

matisation than self-pollination. As stated earlier, variability is the prerequisite for acclimatisation. Therefore, the greater the initial variability, the more the extent of acclimatisation. Life cycle duration is important because an annual would produce several generations by the time a perennial produces one generation. Each generation would produce new gene combinations, thereby facilitating acclimatisation. Mutation plays a role in acclimatisation, but it becomes important only when the period of acclimatisation is very long.

5. **Multiplication and Distribution.** Promising introductions or selections from the introductions may be increased and released as varieties after the necessary trials. Most of the introductions, however, are characterised for desirable traits and are maintained for future use. Such materials are used in crossing programmes and are readily supplied by the bureau on request.

GERMPLASM COLLECTIONS

The sum total of hereditary material or genes present in a species is known as the germplasm of that species. Therefore, *germplasm collection* is collection of a large number of geno types of a crop species and its wild relatives. Germplasm collections are also known as *gene banks* (or *world collections* when they are sufficiently large to include genotypes from all over the world). It should be kept in mind that regular crossing with the wild relatives has played an important role in the evolution of crop plants. Furthermore, germplasm collections furnish the richest source of variability. Crop improvement would ultimately depend upon the availability of this variability to be utilized in breeding programmes.

With modernization of agriculture, large tracts of land are put under pureline varieties of self-pollinated crops and under hybrid varieties of cross-pollinated species. This has led to a gradual disappearance of local or land varieties ('desi' varieties) and open-pollinated varieties—both reservoirs of considerable variability. Cultivation and grazing are gradually destroying many wild species and their breeding grounds. Wild relatives of crops may be eliminated by introduced species of weedy nature or even by the cultivated forms derived from them. *The gradual loss of variability in the cultivated forms and in their wild relatives is referred to as genetic erosion.* This variability arose in nature over a long period of time and, if lost, would not be reproduced in a short period.

Most of the countries are greatly concerned about genetic erosion. The establisment of IBPGR to coordinate germplasm conservation activities throughout the world reflects this concern. Germplasm collections are being made and maintained to conserve as many genotypes as possible. The germplasm collections contain land varieties, various wild forms, primitive races, exotic collections and highly evolved varieties. Some of the important germplasm collections are listed below.

1. Institute of Plant Industry, Leningrad. It has 1,60,000 entries of crop plants.
2. Royal Botanic Gardens, Kew, England. It has over 45,000 entries.
3. Beltsville, U.S.A., maintains germplasm collections of small grain crops.
4. World collections of some of the crops are maintained at the following places.

(i) *Sugarcane.* Canal Point, Florida, U.S.A. and Sugarcane Breeding Institute, Coimbtore (2,800 entries)

(ii) *Groundnut.* Bambey, Senegal (Africa)

(iii) *Potato.* Cambridge, U.K. and Wisconsin, U.S.A.

(iv) *Annual New World Cottons.* Near Tashkent, U.S.S.R.

(v) *Coffee.* Ethiopia (Africa)

(vi) *Sweet Potatoes.* New Zealand.

5. The National Bureau of Plant Genetic Resources, New Delhi, is maintaining large collections of *Sorghum, Pennisetum* wheat, barley, oats, rice, maize and other agricultural and horticultural crops. For example, groundnut collection is maintained at Junagarh, cotton at Nagpur, potato at Simla, tobacco at Rajahmundhry, tuber crops (other than potato) at Trivandrum etc.
6. International Rice Research Institute (IRRI), Los Banos, Philippines, is maintaining 42,000 rice strains and varieties. More than 15,000 entries are maintained at Central Rice Research Institute, Cuttack.
7. The various International Institutes (Chapter 29) are building up and maintaining collections of many species.

Most of the seeds lose viability quickly. Consequently, germplasm collections have to be grown every few years. (1) Growing, harvesting and storing large collections is a costly affair requiring much time, labour, land and money. (2) There is also risk of errors in labelling. (3) The genotypic constitution of the entries may also change, particularly when they are grown in environments considerably different from that to which they are adapted. This is particularly true in case of cross-pollinated species and for local varieties of the self-pollinated species. These difficulties may be considerably reduced by cold storage of seeds. Seeds of most of the plant species can be stored for 10 years or more at low temperatures and low humidity. Thus the entries could be grown every 10 years or so instead of every one or two years. Cold storage facilities are being utilized at Fort Collins, U.S.A and at IRRI, Philippines. NBPGR has developed cold storage facilities for germplasm maintenance ; this is known as *National Germplasm Repository.* However,

low temperature storages of germplasm collections are vulnerable to electricity failures due to normal breakdowns, power cuts, strikes by electricity staff, and (perhaps more importantly) sabotage. In developing countries like India, assured continuous power supply may by one of the major problems of long-term storage. In addition, such storages are likely to become targets of attacks during war. Thus it may be worthwhile to maintain duplicate collections at two separate places, but this would involve considerable additional resources and effort.

It has been suggested that complex-cross populations derived from large number of genetically diverse collections should be maintained as large reservoirs of variability. An International Gene Bank for barley has been established in U.S.A. It accepts F_1 and F_2 seeds from intervarietal crosses from all over the world. The material is made available to the depositors on request for selection of desirable types.

Gene Sanctuaries. It has been proposed that within the centres of origin areas of the greatest diversity should be demarcated and protected from human disturbances. In such areas, the evolutionary potential of the local populations and the environment would be preserved. This would not only preserve variability in these populations, but would also allow evolution to continue and create new types. NBPGR proposes to establish gene sanctuaries in Meghalaya for *Citrus*, and in the North-Eastern Region for *Musa*, *Citrus*, *Oryza*, *Saccharum* and *Mangifera*.

Thus a gene sanctuary may be defined as an area of diversity protected against interference from man. A gene sanctuary conserves the germplasm *in situ*, in the environment where it naturally grows. This not only conserves the germplasm with very little labour and expense, but also permits evolution to proceed on its natural course. This allows the appearance of new gene combinations and new alleles not present in the preexisting population.

EXPLORATION

Explorations are trips for the purpose of collection of various forms of crop plants and their related species. Explorations generally cover those areas that are likely to show the greatest diversity of forms. The centres of origin are such areas and are often visited by various exploration teams. In addition, the land varieties and open-pollinated varieties are also collected. *Exploration is the primary source of all the germplasm maintained in germplasm collections.*

An expedition has to be meticulously planned. It must be based on a sound knowledge of the biosystematics of the genera and species to be collected. The ecology of the vegetation types and the climatology of the region should also be considered. More emphasis should be placed on ecological aspects. A preliminary survey may

be conducted before the collections are actually made. *The job of an exploration is a difficult one : as many diverse types as possible should be collected, and, as far as possible, duplication in the collections should be avoided.* Generally, it is extremely difficult to avoid duplications: this, in turn, leads to overcrowding of germplasm collections. This becomes more difficult because the value of various types, either directly as varieties or as parents in hybridization, cannot be accurately predicted from their appearance, *i.e.*, phenotype.

Indian scientists have undertaken several explorations. In 1955, the Botanical Survey of India sent a team to Bomdila, NEFA. In 1961, an expedition to Central Nepal between Butwal and Pokhra and Muktinath was undertaken. Collections made during this expedition included cultivars of cereals, millets, pulses, mustards. wild species related to wheat, oat, barley, linseed okra and several other crops. Dr. H. B. Singh toured the country extensively in connection with germplasm collection. In addition, explorations were organised in tribal areas of Bihar, Orissa and Andhra Pradesh, in Lahaul and Spiti, in rainfed areas of Madhya Pradesh, Rajasthan and Gujarat, in the North-East Region, in Indonesia and certain parts of U.S.S.R. At present scientists in various coordinated projects of ICAR are required to make collections of land varieties and forward them to the concerned central institutes for evaluation and maintenance.

PURPOSE OF PLANT INTRODUCTION

The main purpose of plant introduction is to improve the plant wealth of the country. The chief objectives of plant introduction may be grouped as follows.

To Obtain an Entirely New Crop Plant. Plant introductions may provide an entirely new crop species. Many of our improtant crops, *e.g.*, maize, potato, tomato, tobacco, soybean etc., are introductions.

To Serve as New Varieties. Sometimes, introductions are released as superior commercial varieties. The Mexican semidwarf wheat varieties, Sonora 64 and Lerma Rojo, semidrawf rice varieties, TN 1 and IR 8, are more recent examples of this type.

To be used in Crop Improvement. Often the introduced material is used in hybridization with local varieties to develop improved varieties. Pusa Ruby tomato was derived from a cross between Meeruty and Sioux, an introduction from U.S.A.

To Save the Crop from Diseases and Pests. Sometimes a crop is introduced into a new area to protect it from diseases and pests. Coffee was introduced in South America from Africa to prevent losses from leaf rust. *Hevea* rubber, on the other hand, was brought to Malaya from South America to protect it from a leaf disease.

For Scientific Studies. Collections of plants have been used in studies on biosystematics, evolution and origin of plant species. N.I. Vavilov developed the concept of centres of origin and that of homologous series in variation from the study of a vast collection of plant types.

Aesthetic Value. Ornamentals, shrubs and lawn grasses are introduced to satisfy the finer sensibilities of man. These plants are used for decoration and are of great value in social life.

SOME IMPORTANT ACHIEVEMENTS OF PLANT INTRODUCTION

The extensive movements of plants from their centres of origin are primarily due to introductions in the prehistoric times. Almost all the countries in the world have obtained some entirely new crops through introduction. In India, introduced materials have been used directly as varieties, released as varieties after selection or used in hybridization programmes. The examples of varieties developed from primary or secondary introductions are too numerous to be listed *in toto*. Some important examples will be cited to illustrate the achievements of plant introduction.



Fig. 2.2.

New Crop Species. The crops introduced in India include such important crops as potato, maize, groundnut, chillies, coffee, *Hevea* rubber, guava, grape, pineapple, papaya etc. Several ornamentals like gulmohar, phlox, salvia, aster etc. are introductions.

Some important recent introductions are : soybean and sugarbeet (in 1960). Introduction of oil palm and ojoba is still in the experimental stages.

Directly Released as Varieties. Semidwarf wheat (*T. aestivum*) varieties, Sonora 64 and Lerma Rojo, were released directly for cultivation. TN 1 rice (*O. sativa*) was introduced from Taiwan and directly released as a variety. Other introduced rice varieties are IR 8, IR 21 and IR 28 from IRRI, Philippines. Other examples include Ridley wheat and Kent oat (*A. sativa*), both from Australia ; Bonneville and Early Badger peas (*P. sativum*) ; Delcrest and Virginia Gold tobacco (*N. tabacum*) ; Bragg, Lee, Clark 63 and Hill Davis soybeans (*G. max*) ; and Sioux tomato (*L. esculentum*), all from USA. Several Introductions of vegetable cowpea, cauliflower, onion, lettuce, watermelon etc. have been released as varieties.

Varieties Selected from Introductions. Many varieties have been developed by selection from introductions. Two varieties of wheat, Kalyan Sona and Sonalika, were selected from introductions from CIMMYT, Mexico. These varieties dominated wheat cultivation in India for about one decade. Other varieties developed through selection are Jamnagar Giant and Improved Ghana bajra ; Pusa Lal and Pusa Sunehari sweet potato, Pusa Basmati, vegetable cowpea, and Japanese White and 40 Days radish etc.

Varieties Developed through Hybridization. Introductions have contributed immensely to the development of crop varieties through hybridization. All the semidwarf wheat varieties are derived from crosses with Mexican semidwarf wheats. All but few semidwarf rice varieties possess the dwarfing gene from Dee-geo-woo-gen, either from TN 1 or IR 8. Thus almost all the semidwarf wheat and rice varieties have been developed from crosses involving introductions. The hybrid maize, jowar (*S. bicolor*) and bajra (*P. americanum*) varieties generally have one parent, the male sterile (or the female) parent, which is either an introduction or derived from an introduction. All the sugarcane varieties have been derived from introduced noble canes, *Saccharum officinarum*.

Other examples of varieties developed through hybridization with introductions are Pusa Ruby tomato obtained from a cross between Meeruti and Sioux ; Pusa Early Dwarf Tomato derived from the cross Meeruti × Red Cloud ; Pusa Kesar carrot, Pusa Kanchan turnip, AF 3 and S 350 bajra etc.

MERITS OF PLANT INTRODUCTION

1. It provides entirely new crop plants.
2. May provide superior varieties directly, after selection or hybridization.
3. Introduction and exploration are the only feasible means of collecting germplasm and to protect variability from genetic erosion.

4. It is a very quick and economical method of crop improvement, particularly when the introductions are released as varieties directly or after simple selection.

5. Plants may be introduced in new disease-free areas to protect them from damage, *e.g.*, coffee and rubber.

DEMERITS OF PLANT INTRODUCTION

The disadvantages of plant introduction are associated with the introduction of weeds, diseases and pests. In the past, several such cases have occurred, some of which are listed below.

Weeds. *Argemone mexicana. Eichornia crassipes* and *Phylaris minor* (gehunsa) are some of the noxious weeds introduced in India. There are many other instances of weeds that were introduced from other countries.

Diseases. Late blight of potato was inroduced from Europe in 1883. Flag smut of wheat was introduced from Australia ; coffee rust came from Ceylon in 1876 ; bunchy top of banana was introduced from Ceylon in 1940. Some other diseases were also introduced in India alongwith plant materials.

Insect Pests. The potato tuber-moth came from Italy in 1900. Woolly aphis of apple and fluted scale of *Citrus* were also introduced in India alongwith plant introductions.

Some introduced species may become noxious weeds in the new habitat. Water hyacinth and *Lantana camara* (kuri) were both introduced as ornamental plants, but they are now noxious weeds.

Some introduced species may disturb the ecological balance in their new home, and may cause serious damage to the ecosystem. *Eucalyptus* sp. introduced from Australia cause a rapid depletion of the subsoil water reserves. Many scientists fear that large scale planting of *Eucalyptus* in India may deplete the subsoil and underground water reserves, which is bound to be detrimental to the ecosystem.

However, most of the cases of introduction of weeds, diseases and insect pests occurred during a period when quarantine was almost nonexistent. At present, plant introductions have to satisfy rigid quarantine laws. They are thoroughly examined for weeds and are fumigated for diseases and pests before their entry is permitted. Thus, at present there is little chance, if any, for the entry of weeds, diseases and pests with plant introductions. But this underlines the necessity for vigilance by the officials responsible for the enforcement of quarantine laws. An error, lapse or carelessness on their part may allow entry to a serious disease, pest or weed species. This may cause considerable economic losses to the nation.

SUMMARY

Crop plants originated from wild species through Mendelian variation, interspecific hybridization or polyploidy. In all these modes of evolution, natural and, perhaps, artificial selections played an important role. The crops and their related wild species show greater diversity in certain areas. Vavilov proposed that these centres of diversity are the *centres of origin* of the crop species. There are eight main centres of origin, *viz.*, China, Hindustan, Central Asia, Asia Minor, Mediterranean, Abyssinia, Central America and South America. It may be more appropriate to call these as centres of diversity rather than centres of origin.

Plant introduction consists of taking a plant species or variety into a new environment where it has not been grown before. Plant movement accompanied the movement of man. In the past, plant introduction was done by invaders, settlers, travellers, traders, pilgrims and explorers. India received a number of plant species through early introductions. At present, plant introduction is the responsibility of the National Bureau of Plant Genetic Resources situated in IARI, New Delhi. The bureau has four substations at Simla, Jodhpur, Kanya Kumari and Akola for evaluation and maintenance of plant collections. The functions of the bureau include introduction, evaluation, multiplication and maintenance of germplasm. Quarantine of the introduced plant materials is also done by the bureau. The bureau supplies germplasm to scientists on request.

The germplasm to be introduced is obtained from other similar agencies in foreign countries or is collected through exploration. The introduced material must be free from weeds, diseases and insect pests. It is the responsibility of the NBPGR to ensure that the introduced material conforms to quarantine regulations. The introduced material must be accompanied by an authentic phytosanitary certificate from the source country.

The introduced material is catalogued, evaluated and, if found suitable, multiplied and released directly as a new variety. Sometimes, new varieties are developed by selection from the introduced material. Performance of some introductions may improve through acclimatisation. Acclimatisation is a process in which genotypes adapted to the new environment increase, while those poorly adapted decrease in proportion. Thus *acclimatisation* is a process of natural selection More often, introductions are used as parents in hybridization programmes. Several important crop varieties have resulted from plant introduction.

Germplasm represents the sum total of the genes present in a species. Germplasm collections are maintained to preserve the genetic variability in crop species and their wild relatives. This is becoming increasingly important due to the ever increasing genetic erosion caused by human activities. Due to *genetic erosion*, wild relatives of crops and the variability present in the crop species is being depleted.

QUESTIONS

1. What are the differences between the following ? (i) Domestication and introduction, (ii) Natural and artificial selection, (iii) Gene bank and gene sanctuary, (iv) Centres of origin and centres of diversity, (v) Selection and acclimatisation, (vi) Primary and secondary introductions, and (vii) Introduction and introgression.
2. Define domestication. What are the important changes that have occurred under domestication ?
3. Describe with the help of suitable examples the sources of variation that have led to the evolution of crop plants.
4. Give the chief contributions of the following scientists : (i) N.I. Vavilov, and (ii) H.B. Singh.

5. List the various centres of origin of cultivated plants. Briefly discuss the concept of centres of origin (primary and secondary). In what way is this concept helpful in plant breeding ?
6. Describe in brief the purpose of plant introduction and outline the various steps involved in plant introduction.
7. What do you understand from quarantine ? Why is it necessary to quarantine plant introductions ? Discuss in detail giving suitable examples.
8. Is genetic variability within a variety essential for : (i) Acclimatisation, (ii) Selection, (iii) Introduction, and (iv) Domestication ? Why ?
9. Describe some important achievements of plant introduction. Discuss the merits and demerits of plant introduction as a breeding method.
10. India has acquired a large plant wealth through plant introductions, and the increased agricultural productivity in the recent years can be traced to some critical introductions. Discuss in detail with the help of suitable examples.
11. Write short notes on the following : (i) Introgressive hybridization, (ii) Centres of origin of crop plants, (iii) Acclimatisation, (iv) Germplasm collection, (v) Exploration, (vi) Microcentres, and (vii) Primary introduction.
12. What is NBPGR ? List the objectives of NBPGR and describe its organisation and functions.

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CHAPTER 3

Modes of Reproduction and Pollination Control

The mode of reproduction determines the genetic constitution of crop plants, that is, whether the plants are normally homozygous or heterozygous. This, in turn, determines the goal of the breeding programme. If the crop plants are naturally homozygous, *e.g.*, as in self-pollinators like wheat, a homozygous line would be desirable as a variety. But if the plants are heterozygous naturally, *e.g.*, as in cross-pollinators like maize, a heterozygous population has to be developed as a variety. Consequently, the breeding methods have to be vastly different for the two groups of crop plants. The knowledge of the mode of reproduction of crop plants is also important for making artificial hybrids. *Production of hybrids between diverse and desirable parents is the basis for almost all the modern breeding programmes.*

MODES OF REPRODUCTION

The mode of reproduction in crop plants may be broadly grouped into two categories, *asexual* and *sexual.*

Asexual Reproduction

Asexual reproduction does not involve fusion of male and female gametes. New plants may develop from vegetative parts of the plant (*vegetative reproduction*) or may arise from embryos that develop without fertilization (*apomixis*).

Vegetative Reproduction. In nature, a new plant develops from a portion of the plant body. This may occur through modified underground and sub-aerial stems, and through bulbils

Underground Stems. The underground modifications of stem generally serve as storage organs and contain many buds. These buds develop into shoots and produce plants after rooting. The examples of such modifications are given below.

Tuber—Potato (*Solanum tuberosum*)

Bulb—Onion (*Allium cepa*), garlic (*Allium sativum*)

Rhizome—Ginger (*Zingiber officinale*), turmeric (*Curcuma domestica*)

Corm—Bunda (*Colocasia antiquorum*), arwi (*Colocasia esculenta*)

Sub-aerial Stems. These modifications include runner, stolon, sucker etc. Sub-aerial stems are used for propagation of mint (*Mentha sp.*), date plam (*Phoenix dactilifera*) etc.

Bulbils. Bulbils are modified flowers that develop into plants directly without formation of seeds. These are vegetative bodies ; their development does not involve fertilization and seed formation. The lower flowers in the inflorescence of garlic naturally develop into bulbils. Scientists are trying to induce bulbil development in plantation crops by culturing young inflorescence on tissue culture media ; it has been successfully done in the case of cardamom.

Artificial Vegetative Reproduction. It is commonly used for propagation of many crop species, although it may not occur naturally in those species. *Stem cuttings* are commercially used for propagation of sugarcane, grapes, roses, etc. *Layering, budding, grafting* and *gootee* are in common use for propagation of fruit trees and ornamental shrubs. Techniques are available for vegetative multiplication through tissue culture in case of many plant species, and attempts are being made to develop the techniques for many other plant species. In many of these species sexual reproduction occurs naturally but for certain reasons vegetative reproduction is more desirable.

Significance of Vegetative Reproduction. The vegetatively reproducing species offer unique possibilities in breeding. A desirable plant may be used as a variety directly regardless of whether it is homozygous or heterozygous. Further, mutant buds, branches or seedlings, if desirable, can be multiplied and directly used as varieties.

Apomixis. *In apomixis, seeds are formed but the embryos develop without fertilization.* Consequently, the plants resulting from them are identical in genotype to the parent plant. In apomictic species, sexual reproduction is either suppressed or absent. When sexual reproduction does occur, the apomixis is termed as *facultative.* But when sexual reproduction is absent, it is referred to as *obligate.* Many crop species show apomixis, but it is generally facultative. The details of apomictic reproduction vary so widely that a confusing terminology has resulted. A simplified classification of apomixis is given below.

Adventive Embryony. In this case, embryos develop directly from vegetative cells of the ovule, such as nucellus, integument, and chalaza. Development of embryo does not involve production of embryo sac. Adventive embryony occurs in mango (*Mangifera indica*), *Citrus*, etc.

Apospory. Some vegetative cells of the ovule develop into unreduced embryo sacs after meiosis. The embryo may develop from egg cell or some other cell of this embryo sac. Apospory occurs in some species of *Hieraceum, Malus, Crepis, Ranunculus,* etc.

Diplospory. Embryo sac is produced from the megaspore, which may be *hapliod* or, more generally, *diploid.* Generally the meiosis is so modified that the megaspore remains diploid. Diplospory leads to *parthenogenesis* or *apogamy.*

1. *Parthenogenesis. The embryo develops from egg cell.* Depending upon whether the embryo sac is haploid or diploid, parthenogenesis is termed as *haploid* or *diploid parthenogenesis.* Haploid parthenogenesis occurs accidentally and has been reported in *Salanum nigrum, Nicotiana, Crepis* and maize. Diploid parthenogenesis occurs in many grasses, *e.g. Taraxacum.*

In many species, *e.g., Nicotiana, Datura,* rice etc., pollen grains may be induced *in vitro* to produce haploid embryos or plantlets. For this, anthers or pollen grains are cultured on a suitable tissue culture medium. This technique offers several possibilities for crop improvement.

2. *Apogamy. In apogamy, synergids or antipodal cells develop into an embryo.* Like parthenogenesis, apogamy may be *haploid* or *diploid* depending upon the haploid or diploid state of the embryo sac. Diploid apogamy occurs in *Antennaria, Alchemilla, Allium* and many other plant species.

Significance of Apomixis. Apomixis is a nuisance when the breeder desires to obtain sexual progeny, *i.e.*, selfs or hybrids. But it is of great help when the breeder desires to maintain varieties. Thus in breeding of apomictic species, the breeder has to avoid apomictic progeny when he is making crosses or producing inbred lines. But once a desirable genotype has been selected, it can be multiplied and maintained through apomictic progeny. This would keep the genotype of the variety intact. Asexually reproducing crop species are highly heterozygous and show severe inbreeding depression. Therefore, breeding methods in such species must avoid inbreeding.

Sexual Reproduction

Sexual reproduction involves fusion of male and female gametes to form a zygote which develops into an embryo. In crop plants, male and female gametes are produced in specialised structure known as flowers.

Flower. A flower usually consists of sepals, petals (or their modifications), *stamens* and/or *pistil.* A flower containing both stamens and pistil is a *perfect* or *hermaphrodite* flowers. If it contains stamens but not pistil, it is known as *staminate* while a *pistillate* flower contains pistil but not stamens. Staminate and pistillate flowers occur on the same plant in a *monoecious* species, such as,

maize, *Colocasia*, castor (*Ricinus communis*), coconut etc. But in *dioecious* species, staminate and pistillate flowers occur on different plants, *e.g.*, papaya, date palm (*Phoenix dactylifera*), pistachio (*Pistacia vera*), hemp (*Cannabis indica*), etc. The male gamete is

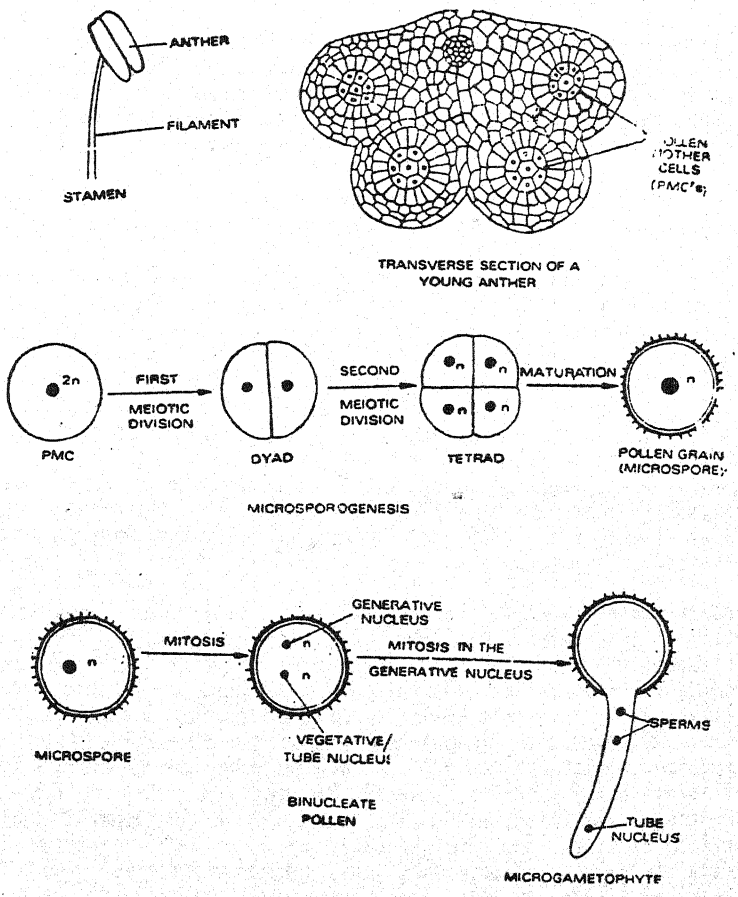


Fig. 3.1. Microsporogenesis and microgametogenesis (a generalised scheme).

produced in stamen, while the pistil produces the female gamete. The male and female gametes are produced in microspores and megaspores, respectively.

Sporogenesis. Production of *microspores* and *megaspores* is known as sporogenesis. Microspores are produced in anthers (*microsporogenesis*), while megaspores are produced in ovules (*megasporogenesis*).

Microsporogenesis. Each anther has four pollen sacs which contain numerous *pollen mother cells* (PMC's). Each PMC undergoes meiosis to produce four haploid cells or *microspores.* This process is known as microsporogenesis (Fig. 3.1). The miorospores mature into pollen grains by thickening of their walls.

Megasporogenesis. Megasporogenesis occurs in ovules which are present inside ovary. A single cell in each ovule differentiates into a *megaspore mother cell.* The megaspore mother cell undergoes melosis to produce four haploid *megaspores.* Three of the megaspores degenerate leaving one functional megaspore per ovule (Fig. 3.2).

Gametogenesis. The production of male and famale gametes in the microspores and megaspores is known as *gametogenesis.*

Microgametogenesis. This refers to the production of male gamete or *sperm.* During the maturation of pollen, the microspore nucleus divides mitotically to produce a *generative* and a *vegetative* or *tube nucleus.* The pollen is generally released in this *binucleate stage.* When the pollen reaches stigma of a flower, it is known as *pollination.* Shortly after pollination, the pollen germinates. The pollen tube enters the stigma and grows through the style. The generative nucleus now undergoes a mitotic division to produce two *male gametes* or *sperms.* The pollen, alongwith the pollen tube, is known as *microgametophyte* (Fig. 3.1). The pollen tube finally enters the ovule through a small pore, *micropyle,* and discharges the two sperms into the embryo sac.

Megagametogenesis. The nucleus of a functional megaspore divides mitotically to produce four or more nuclei. The exact number of nuclei and their arrangement varies considerably from one species to another. In most of the crop plants, megaspore nucleus undergoes three mitotic divisions to produce eight nuclei. Three of these nuclei move to one pole and produce a central *egg cell* and two *synergid cells.* One synergid is situated on either side of the egg cell. Another three nuclei migrate to the opposite pole to give rise to *antipodal cells.* The two nuclei remaining in the centre, the *polar nuclei,* fuse to form the *secondary nucleus.* The megaspore thus develops into a mature *megagametophyte* or *embryo sac. The development of embryo sac from a megaspore is known as megagametogenesis.* The embryo sac generally contains one egg cell, two synergids, three antipodal cells *(all haploid),* and one *diploid* secondary nucleus (Fig. 3.2).

Fertilization. The fusion of one of the two sperms with the egg cell, producing a diploid zygote is known as *fertilization.* The fusion of the remaining sperm with the secondary nucleus leading to the formation of a triploid *primary endosperm nucleus* is termed as *triple fusion.* The zygote divides mitotically to produce a diploid embryo. The primary endosperm nucleus produces endosperm through

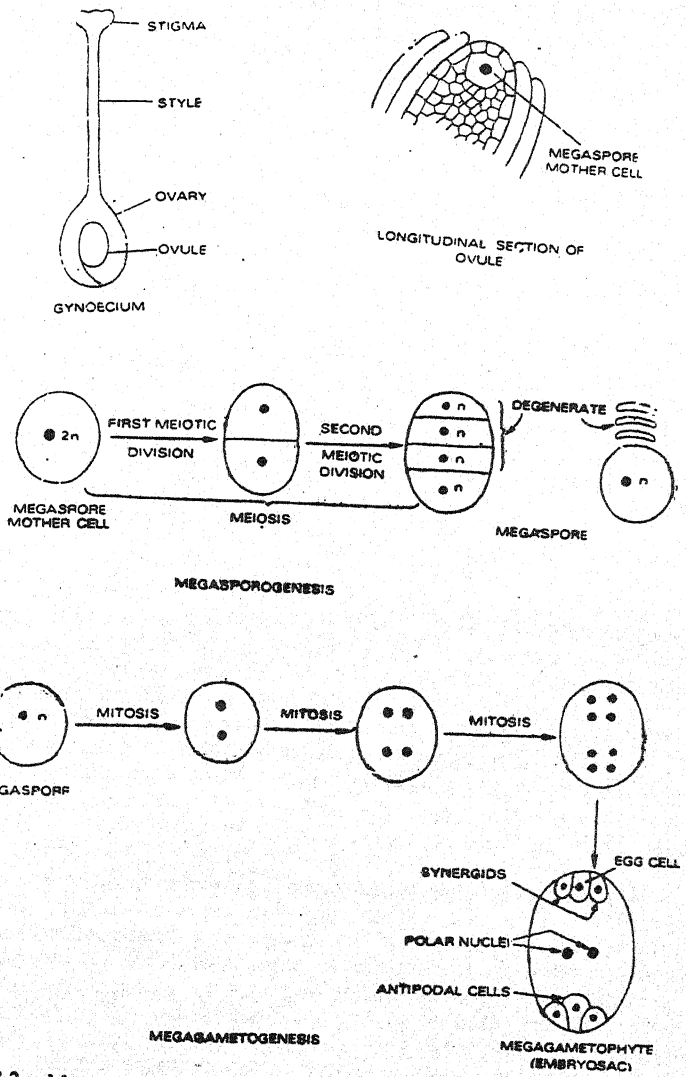


Fig. 3.2. Megasporogenesis and megagametogenesis (a generalised scheme).

repeated mitotic divisions. During seed development, endorsperm provides nutrition to the developing embryo. It may be absorbed completely, *e.g.*, in legumes, or may form the major portion of the seed, *e.g.*, in monocots like maize (*Z. mays*), wheat (*Triticum sp.*) etc., and some dicots, *e.g.*, castor (*R. communis*), *Brassica* sp. etc.

Significance of Sexual Reproduction. Sexual reproduction makes it possible to combine genes from two parents into a single hybrid plant. Recombination of these genes produces a large number of genotypes. This is an essential step in creating variation through hybridization. Almost the entire plant breeding is based on sexual reproduction. Even in asexually reproducing species, sexual reproduction, if it occurs, is used to advantage, *e.g.*, sugarcane, potato, sweet patato etc.

ANTHESIS

In the process of flowering, the first opening of a flower is known as anthesis. The details of anthesis vary from one crop species to another. They are also greatly affected by environmental factors, such as humidity and temperature. A knowledge of the anthesis of a crop is desirable for making successful crosses, and also it largely determines the mode of pollination prevalent in the crop. In case of rice (*O. sativa*), the flower is enclosed by two boat-shaped bracts, *lemma* and *palea.* Petals and sepals are represented by two small sac like *lodicules.* It has six stamens and one ovary. The ovary has two elongated and feathery stigmas. When stigmas become receptive, the lodicules gradually swell and push the lemma and palea apart. Simultaneously, the filaments of stamens elongate pushing the anthers out of the bracts. The anthers dehisce during this process and liberate most of their pollen inside the flower. The remaining pollen grains fall outside. On reaching the stigma, the pollen germinates within few minutes. The pollen tube reaches the embryo sac in about 20 minutes to two hours. Sometimes the anthers may be pushed out before they dehisce, or the pollen may not be functional. In such cases, cross-pollination may take place. In rice, as high as 2-3 per cent cross-pollination may occur.

MODES OF POLLINATION

Pollination refers to the transfer of pollen grains from anthers to stigmas. Pollen from an anther may fall on stigma of the same flower leading to *self-pollination* or *outogamy.* When pollen from flowers of one plant are transmitted to stigmas of flowers of another plant, it is known as *cross-pollination* or *allogamy.* A third situation, *geitonogamy,* results when pollen from a flower of one plant falls on the stigmas of other flowers of the same plant, *e.g.*, in maize (*Z. mays*). The genetic consequences of geitonogamy are the same as those of autogamy.

Self-pollination

Many cultivated plant species reproduce by self-pollination (Table 3.1). Self-pollinated species are believed to have originated from cross-pollinated ancestors. These species, as a rule, must have hermaphrodite flowers. But in most of these species, self-pollination

is not complete and cross-pollination may occur up to 5%. The degree of cross-pollination in self-pollinated species is affected by several factors, *e.g.*, variety, environmental conditions like temperature and humidity, and location. There are various mechanisms that promote self-pollination, which are generally more efficient than those promoting cross-pollination. These machanisms are listed below.

1. *Cleistogamy.* In this case, flowers do not open at all. This ensures complete self-pollination since foreign pollen cannot reach the stigma of a closed flower. Cleistogamy occurs in some varieties of wheats (*Triticum* sp.), oats (*Avena* sp.), barley (*H. vulgare*) and in a number of other grasses.
2. In some species, the flowers open (*chasmogamy*), but only after the pollination has taken place. This occurs in many cereals, such as, wheat, barley, rice and oats. Since the flower does open, some cross-pollination may occur.
3. In crops like tomato (*L. esculentum*) and brinjal (*S. melongena*), the stigmas are closely surrounded by anthers. Pollination generally occurs after the flowers open. But the position of anthers in relation to stigmas ensures self-pollination.
4. In some species, flowers open but the stamens and the stigma are hidden by other floral organs. In several legumes, *e.g.*, pea (*P. sativum*), mung (*V. radiata*), urd (*V. mungo*), soybean (*G. max*) and gram (*C. arietinum*), the stamens and the stigma are enclosed by the two petals forming keel.
5. In a few species, stimgas become receptive and elongate through staminal columns. This ensures predominant self-pollination.

Genetic Consequeness of Self-Pollination. Self-pollination leads to a very rapid increase in homozygosity. Therefore, populations of self-pollinated species are highly homozygous. Self-pollinated species do not show inbreeding depression, but may exhibit considerable heterosis. Therefore, the aim of breeding methods generally is to develop homozygous varieties.

Table. 3.1. Classification of crop species on the basis of their natural mode of pollination

SELF-POLLINATED CROP SPECIES

Cereals and Millets

Wheat (*Triticum aestivum*)
Rice (*Oryza sativa*)
Barley (*Hordeum vulgare*)
Oats (*Avena sativa*)
Foxtail millet (*Setaria italica*)
Ragi (*Eleusine coracana*)

Legumes

Pea (*Pisum sativum*)
Groundnut (*Arachis hypogaea*)
Gram (*Cicer arietinum*)
Mung (*Vigna radiata*)
Urd (*Vigna mungo*)
Cowpea (*Vigna anguiculata*)
Soybean (*Glycine max*)
Sem (*Dolichos lablab*)
Lentil (*Lens esculanta*)
Khesari (*Lathyrus sativus*)
Rajma (*Phaseolus vulgaris*)
Guar (*Cyamopsis tetragonoloba*)
Moth (*Phoseolus aconitifolius*)
Sunnhemp (*Crotalaria juncea*)

Other Crops

Jute (*Corchorus capsularis*)

Vegetables

Tomato (*Lycopersicon esculentum*)
Okra (*Abelmoschus esculentus*)
Lettuce (*Lactuca sativa*)
Brinjal (*Solanum melongena*)
Chillies (*Capsicum annuum*)
Parsnip (*Pastinaca sativa*)
Potato (*Saianum tuberosum*)

Forage Crops

Burr clover (*Medicago hispida*)
Subterranean clover (*Trifolium subterraneum*)
Velvet bean (*Mucuna deeringiana*)
Slender wheatgrass (*Agropyron pauciflorum*)
Several other grasses

Oilseeds

Til (*Sesamum indicum*)
Linseed (*Linum usitatissimum*)

Fruits Trees

Apricot (*Punus armeniaca*)
Nectarine (*Prunus persica*)
Citrus (*Citrus sp.*)
Peach (*Prunus persica*)

CROSS-POLLINATED SPECIES

Cereals

Maize (*Zea mays*)
Rye (*Secale cereale*)
Bajra (*Pennisetum americanum*)

Oilseeds

Some strains of *Brassica campestris*
Sunflower (*Helianthus annus*)
Castor (*Ricinus communis*)
Niger (*Guizotia abyssinica*)

Legumes

Alfala (*Medicago sativa*)
Red clover (*Trifolium pratenss*)
White clover (*Trifolium repens*)
Crimson clover (*Trifolium incarnatum*)
Sweet clover (*Melilotus officinalis*)
Birdsfoot trefoil (*Lotus corniculatus*)

Forage Crops

Ryegrass (*Lolium perenne*)
Timothy grass (*Phleum pratense*)
Smooth bromegrass (*Bromus inermis*)
Johnson grass (*Sorghum halepense*)

Other Crops

Sugarcane (*Saccharum officinarum*)
Some lines of potato (*S. tuberosum*)
Hemp (*Cannabis indica*)
Hops (*Hamulus lupulus*)

Vegetables

Cabbage (*Brassica oleracea*)
Carrot (*Daucus carota*)
Cauliflower (*Brassica oleracea*)
Cucumber (*Cucumis sativus*)
Onion (*Allium cepa*)
Pumpkin (*Curcurbita maxima*)
Radish (*Raphanus sativus*)
Turnip (*Brassica rapa*)
Muskmelon (*Cucurbita moschata*)
Watermelon (*Citrullus vulgaris*)
Squash (*Cucurbita melanosperma*)
Sweet potato (*Ipomoea batatas*)
Others cucurbits (*Cucurbita* sp.)
Beets (*Beta vulgaris*)
Broccoli (*Brassica oleracea*)
Brussels sprouts (*Brassica oleracea*)
Parsley (*Petroselinum hortense*)
Celery (*Apium graveolens*)
Spinach(*Spinacea oleracea*)
Asparagus (*Asparagus officinalis*)
Garlic (*Allium sativum*)
Coriander (*Coriandrum sativum*)

Fruits

Apple (*Pyrus malus*)
Avocado (*Persea americana*)
Mango (*Mangifera indica*)
Pear (*Pyrus communis*)
Blackberries (*Rubus fruticosus*)
Raspberries (*Rubus* sp.)
Walnut (*Juglans regia*)
Chestnut (*Castanea sativa* and *C. Vexa*)
Hazelnut (*Corylus americana* and *C cornuta*)
Banana (*Musa sapientum*)
Cherry (*Prunus avium*)
Date palm (*Phoenix dactilifera*)
Fig (*Ficus carica*)
Coconut (*Cocos nucifera*)
Grapes (*Vitis vinifera*)
Papaya (*Carica papaya*)
Plum (*Prunus divaricata*)
Loquat (*Eriobotrya japonica*)
Strawberries (*Fragaria* sp.)
Almond (*Prunus amygdalus*)
Pistachio nut (*Pistacia vera*)

OFTEN CROSS-POLLINATED CROPS

Jowar (*Sorghum bicolor*)
Cotton (*Gossypium* sp.)
Broad bean (*Vicia faba*)
Jute (*Corchorus olitorius*)
Tobacco (*Nicotiana tabacum* and *N. rustica*)
Pigeon pea or arhar (*Cajanus cajan*)
Rai (*Brassica juncea*)
Brassica campestris var. yellow sarson (yellow sarson)
B. campestris var. toria (toria)
Safflower (*Carthamus tinctorius*)
Triticale (*Triticale hexaploide*)

Cross-Pollination

In cross-pollinating species, the transfer of pollen from a flower to the stigmas of the others may be brought about by wind (*anemophily*), water (*hydrophily*) *or* insects (*entomophily*). Many of the crop plants are naturally cross-pollinated (Table 3.1). In many species, a small amount (up to 5-10 per cent) of selfing may occur. There are several mechanisms that facilitate cross-pollination ; these mechanisms are described briefly.

1. **Dicliny**. *Dicliny* or *unisexuality* is a condition in which the flowers are either staminate (male) or pistillate (female).

(a) *Monoecy*. Staminate and pistillate flowers occur in the same plant, either in the same inflorescene, *e.g.*, castor, mango, (*Mangifera indica*), banana (*Musa sapientum*) and coconut, or in separate inflorescences, *e.g.*, maize. Other monoecious species are cucurbits (*Cucurbita* sp.), walnut (*Juglans regia*), chestnut, strawberries (*Fragaria* sp.,) rubber (*Hevea* sp.), grapes (*Vitis vinifera*) and cassava (*M. utilissima*).

(b) *Dioecy*. The male and female flowers are present on different plants, *i.e.*, the plants in such species are either male or female, *e.g.*, papaya (*C. papaya*), date (*Phoenix dactilifera*), hemp (*Cannabis indica*), asparagus (*Asparagus officinalis*), and spinach (*Spinacea oleracea*). In general, the sex is governed by a single gene, *e.g.*, asparagus and papaya. In some cases, there are hermaphrodite plants in addition to males and females, and a number of intermediate forms may also occur.

2. **Dichogamy**. Stamens and pistils of hermaphrodite flowers may mature at different times facilitating cross-pollination.

(a) *Protogyny*. In crop species like bajra, pistils mature before stamens.

(b) *Protandry*. In crops like maize and sugarbeets (*B. vulgaris*), stamens mature before pistils.

3. In lucerne or alfalfa (*M. sativa*), stigmas are covered with a waxy film. The stigma does not become receptive until this waxy film is broken. The waxy membrane is broken by the visit of honey bees which also effect cross-pollination.

4. A combination of two or more of the above mechanisms may occur in some species. This improves the efficiency of the system in promoting cross-pollination. For example, maize exhibits both monoecy and protandry.

5. **Self-Incompatibility**. *It refers to the failure of pollen from a flower to fertilize the same flower or other flowers on the same plant.* Self-incompatibility is of two types : *sporophytic* and *gametophytic*. In both the cases, flowers do not set seed on selfing. Self-incompatibility is common in several species of *Brassica* (mustard, rai, cauliflower, etc.), some species of *Nicotiana*, radish, rye and many grasses. It is highly effective in preventing self-pollination.

6. **Male Sterility.** *Male sterility refers to the absence of functional pollen grains in otherwise hermaphrodite flowers.* Male sterility is not common in natural populations. But it is of great value in experimental populations, particularly in the production of hybrid seed. Male sterility is of two types : *genetic* and *cytoplasmic.* Cytoplasmic male sterility is termed *cytoplasmic-genetic* when restorer genes are known. In view of the importance of self-incompatibility and male sterility, a more detailed discussion on them follows later.

Genetic Consequences of Cross-Pollination. Cross-pollination preserves and promotes heterozygosity in a population. Cross-pollinated species are highly heterozygous and show mild to severe inbreeding depression and a considerable amount of heterosis. The breeding methods in such species aim at improving the crop species without reducing heterozygosity to an appreciable degree. Usually, hybrid or synthetic varieties are the aim of breeder wherever the seed production of such varieties is economically feasible.

Often Cross-Pollinated Species

In many crop plants (Table 3.1), cross-pollination often exceeds 5 per cent and may reach 30 per cent. Such species are generally known as *often cross-pollinated species, e.g.,* jowar, cotton (*Gossypium sp.*), arhar (*Cajanus cajan*), safflower (*C. tinctorius*) etc. The genetic architecture of such crops is intermediate between self-pollinated and cross-pollinated species. Consequently, in such species breeding methods suitable for both of them may be profitably applied. But often hybrid varieties are superior to others.

DETERMINATION OF THE MODE OF REPRODUCTION IN A SPECIES

The first step in determining the mode of reproduction of a species is to critically examine its flowers. Mechanisms like dioecy, monoecy, protogyny, protandry and cleistogamy are easily detected ; they clearly indicate the mode of pollination.

The second step consists of isolating single plants and recording seed set under isolation. Space isolation, *i.e.,* individual plants grown at sufficient distance to prevent cross-pollination, is preferable to isolation by bags or cages. Isolation by bags or cages may create an environment unfavourable for pollination and seed set. Failure to set seed in isolation proves the species to be cross-pollinated. However, setting of seeds is only indicative of self-pollination.

Finally, the effects of selfing (inbreeding) on the vigour of plants should be studied. Loss in vigour due to inbreeding is common in cross-pollinators, but self-pollinators show no inbreeding depression.

DETERMINATION OF THE AMOUNT OF CROSS-POLLINATION IN A SPECIES

The amount of cross-pollination is determined by planting two strains of the species in a mixed stand. One strain is homozygous for a dominant character, preferably, an easily recognisable seed or seedling character, while the other strain has the recessive form of the character. Each plant of the recessive strain is surrounded by plants of the dominant strain to provide abundant pollen. The seeds from the plants of recessive strain are harvested. The percentage of seeds carrying the dominant allele represents the percentage of crosspollination in the species.

The frequency of cross-pollination varies greatly with the variety, weather conditions and location. For example, in a study on safflower, the estimates of outcrossing in different varieties grown in the same year at the same location ranged from 0–8.7%. Similarly, the amount of cross-pollination in a single variety grown at several locations varied from 1.3 to 9.8 per cent. As a result, such a study should include several varieties and locations, and should be conducted for two or more years. In view of this, the information on many crops may be regarded as incomplete.

Whether a species is apomictic may be determined by crossing a recessive strain (used as female) to a dominant one (used as male). If a sufficiently high frequency of recessive offspring are recovered in the progeny, the species is most likely apomictic. But every precaution should be taken to avoid self-pollination while making the crosses.

RELEVANCE OF THE MODE OF REPRODUCTION TO PLANT BREEDING

The modes of reproduction and pollination are very important in plant breeding because they determine (1) the genetic constitution of a species, (2) the ease in pollination control, and (3) the stability of varieties after release.

Genetic Constitution. The cross-pollinated species are highly heterozygous, and generally show loss in vigour on inbreeding. The self-pollinated species, on the other hand, consist of homozygous individuals of similar or different genotypes. Self-pollinators do not show inbreeding depression. The asexually reproducing crops are similar to the cross-pollinated species. Primarily because of the effects of inbreeding, the breeding approaches and the varieties developed are different for the three different groups of crops. In self-pollinating species, the varieties are homozygous, and often a variety consists of plants of a single genotype. In the cross-pollinating species, on the other hand, the varieties are highly heterozygous and consist of one (hybrid varieties) or many (open-pollinated and synthetic varieties) genotypes. The asexually reproducing species present an added advantage since asexual progeny from any desirable plant at any stage of the breeding programme may be

directly used as a variety. In such crops, the varieties are clones, *i.e.*, asexual progeny of single plants.

Facility in Controlled Pollination. Breeding methods invariably depend upon some system of controlled mating. In most of the breeding schemes, selected plants or strains have to be crossed. The ease or the difficulty in making these crosses and the precautions necessary to ensure or prevent selfing largely depend upon the floral structure and the mode of pollination of the species. For example, in self-pollinating crops selfing occurs naturally, while in cross-pollinating species flowers have to be hand-pollinated and protected from foreign pollen.

Stability of Varieties after Release. Since in self-pollinators varieties are homozygous and natural cross-pollination is negligible, they are fairly stable in their genetic constitution. Farmers may plant the same seed for several years if precaution is taken to avoid off-types due to mechanical mixtures. Similar is the case with asexually reproducing species. But in cross-pollinating species, sufficient precaution has to be taken to avoid contamination by foreign pollen. In the case of hybrid varieties, farmers have to replace the seed every year due to severe inbreeding depression in the subsequent generations. Even in case of synthetic varieties, the seed should be replaced every few years. But asexually reproducing crops have a problem of their own. They are often infected by one or the other virus which, unlike sexually reproducing species, is transmitted from one generation to the next. This leads to reduced vigour and yields, and necessitates the replacement of seeds every few years.

MECHANISMS OF POLLINATION CONTROL IN CROP PLANTS

There are several natural mechanisms that control the mode of pollination in crop plants. These mechanisms have been briefly described earlier while discussing self- and cross-pollination. Two of these mechanisms, self-incompatibility and male sterility, are of special significance because of their utilization in hybrid seed production. Therefore, these mechanisms will be considered in some detail.

Self-Incompatibility

More than 300 species belonging to 20 families of angiosperms show self-incompatibility. Self-incompatible pollen grains fail to germinate on the stigma. If some pollen grains do germinate, pollen tubes fail to enter the stigma. In many species, the pollen tubes enter the style, but they grow too slowly to effect fertilization before the flower drops. Sometimes, fertilization is effected, but the embryo degenerates at a very early stage. Self-incompatibility appears to be a biochemical reaction, but the precise nature of these reactions is not clearly understood. The genetic control of incompatibility reactions is relatively simple. Lewis (1954) has suggested various classifications of self-incompatibility ; a relativelys imple classifica-

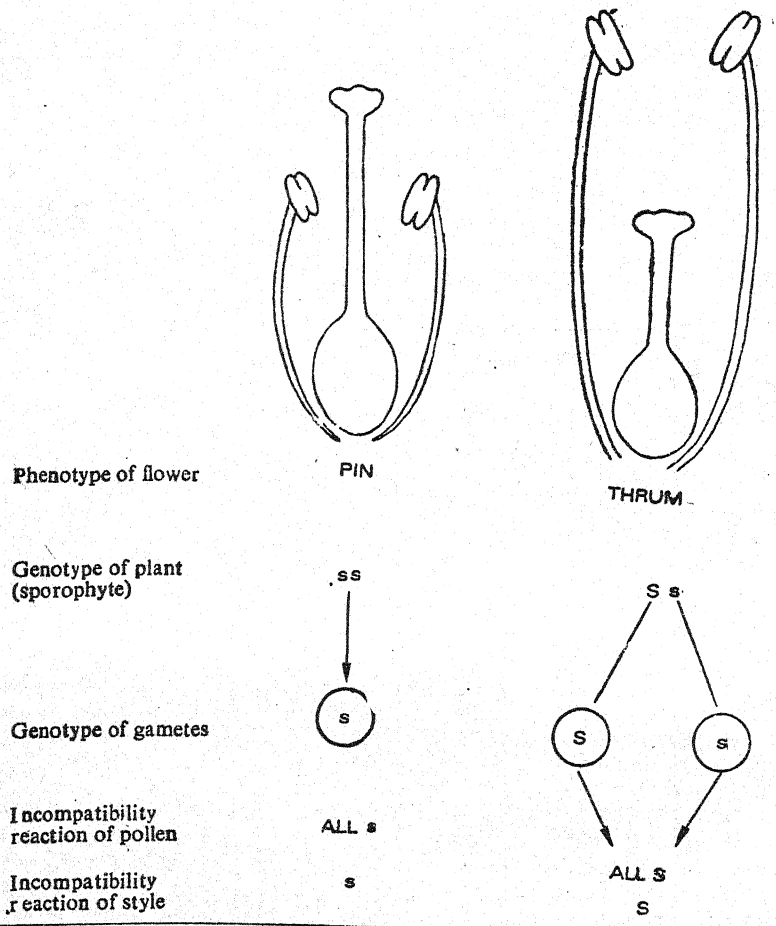
tion is as follows : (1) heteromorphic system, (2) homomorphic system, (2a) gametophytic control, and (2b) sporophytic control.

Heteromorphic System. In this system, flowers of different incompatibility groups are different in morphology. For example, in *Primula* there are two types of flowers, pin and thrum. *Pin* flowers have long styles and short stamens, while *thrum* flowers have short styles and long stamens. This situation is referred to as *distyly*. *Tristyly* is known in some plant species, *e.g.*, *Lythrum* ; in such cases, the style of a flower may be either short, long or of medium length. In the case of distyly, the only compatible mating is between pin and thrum flowers. This characteristic is governed by a single gene *s*, *Ss* producing thrum and *ss* producing pin flowers. The incompatibility reaction of pollen is determined by the genotype of the plant producing them. Allele *S* is dominant over *s* The incompatibility system, therefore, is *heteromorphic-sporophytic*. Pollen grains produced by pin flowers would all be *s* in genotype as well as in incompatibility reaction. But pollen produced in thrum flowers would be of two types genotypically, *S* and *s*, but all of them would be S phenotypically. The mating between pin and thrum plants would produce *Ss* and *ss* progeny in equal frequencies (Fig. 3.3). This system is of little importance in crop plants ; it occurs in sweet potato and buckwheat.

Homomorphic System. In the homomorphic system, incompatibility is not associated with morphological differences among flowers. The incompatibility reaction of pollen may be controlled by the genotype of the plant on which it is produced (*sporophytic control*) or by its own genotype (*gametophytic control*).

Gametophytic System. Gametophytic incompatibility was first described by East and Mangelsdorf in 1925 in *Nicotiana sanderae*. *The incompatibility reaction of pollen is determined by its own genotype, and not by the genotype of the plant on which it is produced* (Fig. 3.4). Generally, the incompatibility reaction is determined by a single gene having multiple alleles, *e.g.*, *Trifolium*, *Nicotiana*, *Lycoperscion*, *Solanum*, *Petunia* etc. Sometimes, polyploidy may lead to a loss of incompatibility due to competition between the two *S* alleles in diploid pollen. Irradiation of pollen or buds with X-rays or gamma-rays temporarily suppresses the incompatibility reaction, and thus allows the pollen tube to grow through incompatible style. In some species, *e.g*, *Phalaris*, *Physalis* etc., two loci (*S* and *Z*) govern incompatibility, while in some others, *e.g.*, *Beta vulgaris* and *Papaver*, three loci are involved. In these cases, polyploidy does not affect the incompatibility reaction. Pollen tube grows very slowly in the style containing the same *S* allele as the pollen, and fails to effect fertilization. Therefore, all the plants are heterozygous at the *S* locus. In a single gene system, there are three types of matings :

(i) Fully incompatible, *e.g.*, $S_1S_2 \times S_1S_2$



Mating		*Progeny*	
Phenotype	*Genotype*	*Genotype*	*Phenotype*
Pin × pin	*ss* × *ss*	Incompatible Mating	
Pin × thrum	*ss* × *Ss*	1 *Ss* : 1 *ss*	1 Thrum : 1 pin
Thrum × pin	*Ss* × *ss*	1 *Ss* : 1 *ss*	1 Thrum : 1 pin
Thrum × thrum	*Ss* × *Ss*	Incompatible Mating	

Fig. 3.3. Heteromorphic-sporophytic system of incompatibility. Relative position of stigma and anthers is governed by a single gene *S*/*s*. The incompatibility reaction of pollen is governed by the genotype of the sporophyte on which it is produced, while that of style is controlled by its own genotype. Allele *S* is dominant over *s* in determining incompatibility as well as the length of style. Pollen of an incompatibility group would not be able to grow normally in the style of the same incompatibility group and would be unable to effect fertilization. Such a mating is said to be incompatible.

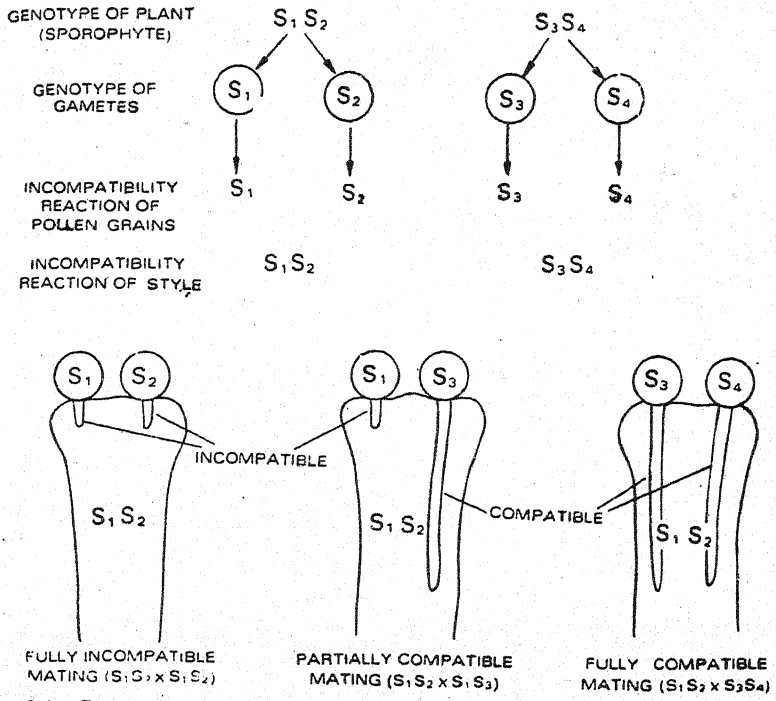


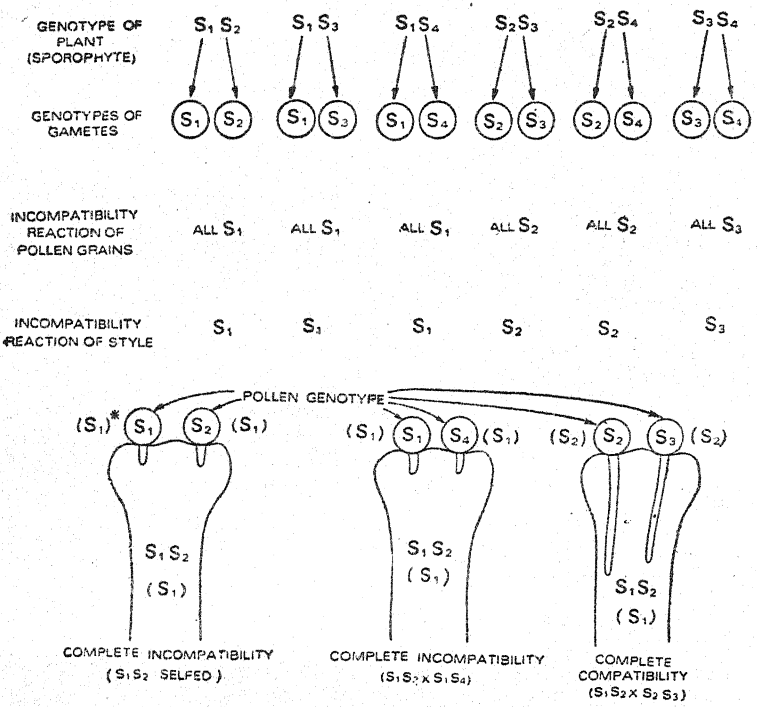
Fig. 3.4. Gametophytic system of incompatibility. The incompatibility reaction of pollen is governed by the genotype of pollen itself. The incompatibility reaction of style is determined by its own genotype and generally the two alleles do not show dominance, that is, they show codominance. The self-incompatibility gene *S* has multiple alleles.

(ii) Fully compatible, *e.g.*, $S_1S_2 \times S_3S_4$

(iii) Partially (*i.e.*, 50% of the pollen) compatible, *e.g.*, $S_1S_2 \times S_2S_3$.

In some cases, an allele for self-fertility, S_f, is found. Pollen carrying the S_f allele does not show incompatibility reaction. Thus in a plant with the genotype S_fS_1, selfing produces S_fS_f and S_fS_1 progeny. Mutations for S_f allele may be induced by irradiating the pollen used for self-pollination. There is another allele, S_F, which retards the growth of S_f pollen tubes, thus enforcing self-incompatibility. The gametophytic system is found in pineapple (2 locus), ryegrass (2 locus), diploid coffee, diploid clovers (*Trifolium sp.*) etc.

Sporophytic System. In the sporophytic system also, the self-incompatibility is governed by a single gene, *S*, with multiple alleles ; more than 30 alleles are known in *Brassica oleracea.* In general, the number of *S* alleles is considerably larger in the gametophytic than in the sporophytic system. *The incompatibility reaction of*



The letters within parenthesis, *e.g.*, (S_1) and (S_2) denote the incompatibility reactions of pollen grains and styles. A complete dominance is assumed.

Fig. 3.5. Sporophytic system of incompatibility. Incompatibility reaction of pollen grains is controlled by the genotype of the plant (sporophyte) on which they are produced, while that of style is governed by its own genotype. For simplicity, it is assumed that the incompatibility alleles (*S* alleles) show complete dominance in the manner $S_1 > S_2 > S_3 > S_4 \ldots$ etc. The actual situation is much more complicated and several different types of allelic relationships are encountered.

pollen is governed by the genotype of the plant on which the pollen is produced, and not by the genotype of the pollen (Fig. 3.5). It was first reported by Hughes and Babcock in 1950 in *Crepis foetida*, and by Gerstel in *Parthenium argentatum* (in the same year). In the sporophytic system, the *S* alleles may exhibit dominance, individual action (codominance) or competition. Consequently, there may be many complex incompatibility relationships. Lewis has summarised the following characteristics of this system :

1. There are frequent reciprocal differences.
2. Incompatibility can occur with the female parent.
3. A family can consist of three incompatibility groups.
4. Homozygotes are a normal part of the system.
5. An incompatibility group may contain two genotypes.

The sporophytic incompatibility is found in radish (*R. sativus*), diploid *Brassica* crops and *Sinapis*. In many cases, different *S* alleles vary in their activity leading to varrying degrees of self-incompatibility, *e.g.*, *B. oleracea*. Polygenes (*modifying genes*) are known to increase as well as decrease the activities of *S* alleles both in the gametophytic as well as sporophytic systems.

Mechanism of Self-Incompatibility

The mechanism of self-incompatibility is quite complex and is poorly understood. The various phenomena observed in self-incompatible matings are grouped into three broad categories : (1) pollen-stigma interaction, (2) pollen tube-style interaction, and (3) pollen tube-ovule interaction.

Pollen-Stigma Interaction. These interactions occur just after the pollen grains reach the stigma and generally prevent pollen germination. At the time they reach stigma, pollen grains generally have two nuclei in the gametophytic system, while they have three nuclei in the sporophytic system. This was once considered to be the basis for the two incompatibility systems, but the available evidence indicates otherwise. However, the structure of stigmatic surface appears to be definitely involved in the differences between two systems. In the *gametophytic system*, the stigma surface is plumose having elongated receptive cells and is commonly known as 'wet' stigma. The pollen grains generally germinate on reaching the stigma, and the incompatibility reaction occurs at a later stage. There are clear-cut serological differences among the pollen grains with different *S* genotypes ; such differences have not been observed in the sporophytic system.

In the *sporophytic system*, the stigma is papillate and dry, and is covered with a hydrated layer of proteins known as 'pellicle'. There is evidence that the pellicle is involved in incompatibility reaction. There are striking differences in the stigma antigens related to the *S* allele composition. Within few minutes of reaching the stigmatic surface, the pollen releases an exine exudate which is either protein or glycoprotein in nature. This exudate induces immediate callose formation in the papilae (which are in direct contact with the pollen) of incompatible stigma. Often, callose is also deposited on the young protruding pollen tubes preventing further germination of the pollen. Thus in the sporophytic system, stigma is the site of incompatibility reaction ; once the pollen tube crosses the stigmatic barrier, there is no further inhibition of the pollen tube growth. In the homomorphic sporophytic system, the incompatibility reaction of pollen is probably due to the deposition of some compounds from anther tapetum on to the pollen exine.

Pollen Tube-Style Interaction. In most cases of the gametophytic system, pollen grains germinate and pollen tubes penetrate the stigmatic surface. But in incompatible combinations, the growth of pollen tubes is retarded within the stigma, *e g.*, in *Oenothera*, or a little later in the style, *e.g.*, in *Petunia*, *Lycopersicon*, *Lilium* etc. In the

latter case, there is a cessation of protein and polysaccharide synthesis in the pollen tubes, which leads to the degeneration of tube wall and the bursting of pollen tube.

Pollen Tube-Ovule Interaction. In some cases, *e.g.*, *Theobromo cacao*, pollen tubes reach the ovule and effect fertilization. However, in incompatible combinations, embryos degenerate at an early stage of development.

Relevance of Self-Incompatibility in Plant Breeding

Self-incompatibility effectively prevents self-pollination. As a result, it has a profound effect on breeding approaches and objectives. These are discussed here in some detail.

1. In self-incompatible fruit trees, it is necessary to plant two cross-compatible varieties to ensure fruitfulness. Further, cross-pollinalion may be poor in adverse weather conditions reducing fruit set. Therefore, it would be desirable to develop self-fertile forms in such cases.
2. Some breeding schemes, *e.g.*, development of hybrid varieties, etc., initially require some degree of inbreeding. Although sib-mating leads to inbreeding, but for the same degree of inbreeding it takes twice as much time as selfing. Further, for the maintenance of inbred lines selfing would be necessary.
3. Self-incompatibility may be used in hybrid seed production. For this purpose, (1) two self-incompatible, but cross-compatible, lines are interplanted ; seed obtained from both the lines would be hybrid seed. (2) Alternatively, a self-incompatible line may be interplanted with a self-compatible line. From this scheme, only the seed from self-incompatible line would be hybrid. (3) Schemes for the production of double cross and triple cross hybridse hav also been proposed and their feasibility has been demonstrated in the case of brassicas.

The gametophytic system has been used, to a limited extent, for hybrid seed production in clover, *Trifolium* (Leguminosae). In Solanaceae, the cultivated species are generally self-fertile, and self-incompatibility is confined to wild species. The sporophytic system has been exploited for hybrid seed production in brassicas (Cruciferae), primarily by the Japanese seed companies. In Compositae, another economically important family showing sporophytic self-incompatibility, the cultivated varieties are generally self-fertile.

The use of self-incompatibility in hybrid seed production is hampered by several problems. (1) Production and maintenance of inbred lines by hand pollination is tedius and costly. (2) This raises the cost of hybrid seed. (3) Continued selfing leads to a depression in self-incompatibility, and it unintentionally, but unavoidably, selects for self-fertility. (4) In the gametophytic system, continued inbreeding gives rise to new incompatibility reactions, which may limit the usefulness of the inbreds as parents. (5) Evironmental

factors, *e.g.*, high temperature and high humidity etc., reduce or even totally overcome self-incompatibility reaction, leading to a high (30% or more) proportion of selfed seed. (6) Bees often prefer to stay within a parental line, particularly when the parental lines differ morphologically. This, in turn, increases the proportion of selfed seed. (7) Transfer of *S* alleles from one variety or, more particularly, species into another variety or species is tedius and complicated. This has prevented the use of self-incompatibility in hybrid seed production in Solanaceae and Compositae.

Elimination of Self-Incompatibility

In many cases, self-fertile forms will be highly desirable and, in such cases, it would be useful to eliminate self-incompatibility. (1) In the case of single-locus gametophytic system, incompatibility may be eliminated by *doubling the chromosome nnmber, e.g.*, in potato diploidization leads to self-incompatibility. (2) *Isolation of self-fertile* (S_f) *mutations* is a very useful tool in the elimination of self-incompatibility. Flower buds are generally irradiated at the PMC stage, and pollen from these buds is used to pollinate flowers with known *S* alleles. Generally, selection for S_f alleles is much more complicated in the sporophytic system than in the gametophytic system due to the temporary loss in incompatibility and pseudofertility in the case of the former. In *Oenothera*, S_f mutations occur spontaneously at the rate of 10^{-6}, and the rate of induction with X-rays is 1.6×10^{-6}/r unit. Lastly, (3) *self-compatibility alleles may be transferred* from related species or varieties of the same species, if available, through a backcross programme.

Overcoming Self-Incompatibility

In many situations, *e.g.*, during the production of inbreds for use as parents in hybrid seed production, it is essential that temporary self-fertility is achieved in a manner so that self-incompatibility is fully functional in the selfed progeny. Such self-fertility is known as *pseudofertility* and is achieved by temporarily suppressing the incompatibility reaction using one of the following techniques.

Bud Pollination. Bud pollination means application of mature pollen to immature nonreceptive stigma, generally 1-2 days prior to the anthesis of flowers. This is the most practicable and successful method both in the gametophytic and sporophytic systems. In some cases, application of the fluid from mature stigmas may improve the success of bud pollination.

Surgical Techniques. Removal of the stigmatic surface, the whole of stigma or a part or whole of the style may permit an otherwise incompatible mating. Removal of the stigma is very useful in the sporophytic system, *e.g.*, *Brassica*, while removal of the style is helpful in some cases of gametophytic incompatibility, *e.g.*, *Petunia*. In *Petunia*, the whole of the style may be removed and the pollen grains may be directly dropped on to the ovules in the ovarian cavity.

End-of-Season Pollination. In some species, the degree of incompatibility is reduced towards the end of the flowering season or in mature plants. But there are controversial reports on the usefulness of this technique.

High Temperature. In some species, *e.g.*, *Trifolium*, *Lycopersicon*, *Brassica*, *Oenothera* etc., exposure of pistils to temperatures upto 60°C induces pesudo-fertility.

Irradiation. In the single-locus gametophytic system, *e.g.*, in Solanaceae, acute irradiation with X-rays or gamma-rays induces a temporary loss of self-incompatibility.

Grafting. Grafting of a branch onto another branch of the same plant or of another plant is reported to reduce the degree of self-incompatibility in *Trifolium pratense.* There is only one report on this phenomenon, and the machanism of this reduction is not known.

Double Pollination. In some species, self-incompatible matings become possible when incompatible pollen is applied as a mixture with a compatible pollen, or it is applied after pollination with a compatible pollen.

Other Techniques. A number of other techniques have been tried with varying degrees of success, but they are not commonly used. These techniques are : treatment of flowers with carbon monooxide, injecting styles with immunosuppressants, application of electrical potential difference of about 100 V between the stigma and pollen grains, treatment of pistil with phytohormones and with protein synthesis inhibitors, and steel brush pollination.

MALE STERILITY

Male sterility is characterised by nonfunctional pollen grains, while famale gametes function normally. It occurs in nature sporadically, perhaps due to mutation. Male sterility is classified into three groups : (1) genetic, (2) cytoplasmic, and (3) cytoplasmic-genetic.

Genetic Male Sterility

Genetic male sterility is ordinarily governed by a single recessive gene, *ms*, but dominant genes governing male sterility are also known, *e.g.*, in safflower. Male sterility alleles arise spontaneously or may be artificially induced. A male sterile line may be maintained by crossing it with heterozygous male fertile plants. Such a mating produces 1 : 1 male sterile and male fertile plants (Fig. 3.6).

Utilization in Plant Breeding. Genetic male sterility may be used in hybrid seed production. The progeny from *ms ms* × *Ms ms* crosses are used as female, and are interplanted with a homozygous male

fertile (*Ms Ms*) pollinator. The genotypes of the *ms ms* and *Ms ms* lines are identical except for the *ms* locus, *i.e.*, they are isogenic, and are known as male sterile (A) and maintainer (B) lines, respectively. The female line would, therefore, contain both male sterile and male fertile plants ; the latter must be identified and removed before pollen shedding. This is done by identifying the male fertile plants in seedling stage either due to the pleiotropic effect of the *ms* gene or due to the phenotypic effect of a closely-linked gene. Pollen dispersal from the male (pollinator) line should be good for a satisfactory seed set in the female line. However, generally pollen dispersal is poor and good, closely-linked markers are rare. Roguing of male fertile plants from the female line is costly as a result of which the cost of hybrid seed is higher. Due to these difficulties, genetic male sterility has been exploited commercially only in a few countries. In USA, it is being successfully used in castor. In India, it is being used for hybrid seed production of arhar (*C. cajan*) by some private seed companies, *e.g.*, Maharashtra Hybrid Seed Co. Ltd., India, produced and sold 50 Q seed of a hybrid variety of arhar. Suggestions have been made for its use in several other crops, *e.g.*, cotton, barley, tomato, sunflower, cucurbits etc., but it is not yet practically feasible.

Inheritance of Male Sterility

Parents — *ms ms* × *Ms Ms*
(Male sterile) (Male fertile)
↓
F_1 — *Ms ms*
(Male fertile)
↓
F_2 — 1 *Ms Ms*, 2 *Ms ms*, 1 *ms ms* — Ratio 3 : 1
Male fertile Male sterile

Maintenance of A Male Sterile Line

♀ Male sterile strain A	♂ Male fertile strain B	Cross the male serlie line with the male fertile sib line
ms ms × *Ms Ms*		
↓		
♀ *ms ms* ×	*Ms Ms* ♂	The F_1 (*Ms ms*) is back-
Male sterile (line A)	Male fertile (line B)	crossed with the male sterile line A
↓		
♂ 1 *Ms ms* : 1 *ms ms* ♀		Maintained by sib mating.
Male fertile × Male sterile		Seed from the male sterile
(Seed not harvested)	(Seed harvested)	plants only is harvested.
↓		
♂ 1 *Ms ms* : 1 *ms ms* ♀		Maintained by sib mating
Male fertile × Male sterile		Seed from the male sterile
(Seed not harvested)	(Seed harvested)	plants only is harvested.
↓		
Maintained indefinitely		

Fig. 3.6. Inheritance of genetic male strarility. Male sterility is produced by a recessive nuclear gene designated as *ms* ; the dominant allele *Ms* produces male fertility. During the maintenance of male stevile lines, sibmating is achieved through natural pollination.

Cytoplasmic Male Sterility

This type of male sterility is determined by the cytoplasm (Fig. 3.7). Since the cytoylasm of a zygote comes primarlily from egg cell, the progeny of such male sterile plants would always be male sterile. Cytoplasmic male sterility is known in many plant species, some of which are crop plants (Table 3.2). Cytoplasmic male sterility may be transferred easily to a given strain by using that strain as a pollinator (recurrent parent) in the successive generations of a backcross programme (Fig. 3.8). After 6-7 backcrosses, the nuclear genotype of the male sterile line would be almost identical to that of the recurrent pollinator strain. The male sterile line is maintained by crossing it

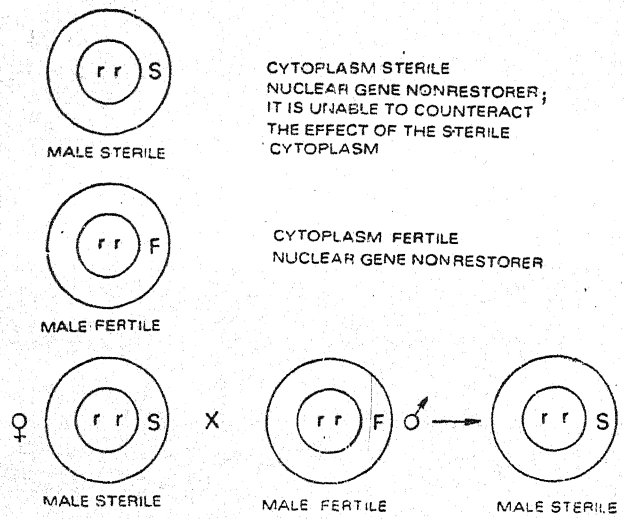


Fig 3.7. Cytoplasmic male sterility. Male sterility is caused by the cytoplasm (depicted by S). The F cyloplasm is the normal cytoplasm. If a restorer gene (the dominant allele of *r*) becomes available, the male sterility will be included in the cytoplasmic-genetic group. This type of male sterility shows strict cytoplasmic inheritance.

with the pollinator strain used as the recurrent parent in the backcross programme since its nuclear genotype is identical with that of the male sterile line. Such a male fertile line is known as the *maintainer line* or *B line* as it is used to maintain the male sterile line. The *male sterile line* is also known as the *A line*. There is considerable evidence that the gene or genes conditioning cytoplasmic male sterility, particularly in maize, reside in mitochondria, and may be located in a plasmid like element.

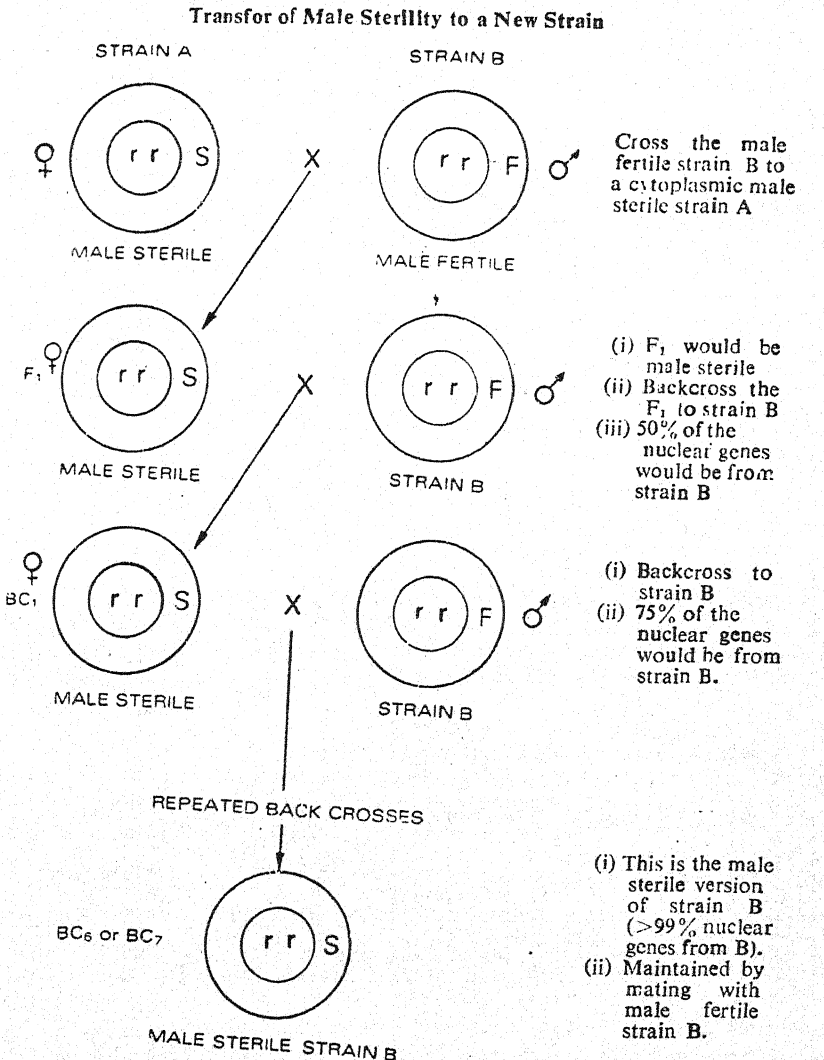


Fig. 3.8. Transfer of the male sterile ceytoplasm from strain A to strain B. Strain A is used as the non-recurrent parent in the backcross programme with strain B. After 6-7 backcrosses, the progeny will be similar to strain B in their nuclear genotype. The strain B serves as the maintainer of the new male sterile strain.

Table 3.2. Cytoplasmic and cytoplasmic-genetic male sterility in some important crop plants.

Crop species (nucleus)	*Cytoplasm*	*Restorer genes*	*Remarks*
Maize, (*Zea mays*)	cms-C (*Z. mays*)*	One (Rf_3)**	Spontaneous reversion relatively high
	cms-S (*Z. mays*)*	One (Rf_4)**	Commercially used
	cms-T (*Z. mays*)*	Two (Rf_1, Rf_2)**	Most commonly used, slightly retards growth and yield (2-4%), susceptibility to *Helminthosporium* leaf blight
Nicotiana tabacum	*Nicotiana debneyi* *Nicotiana megalosiphon* *Nicotiana bigelovii*		Poor growth, floral abnormalities, commercially not used.
Triticum aestivum	*Triticum timopheevii* *Aegilops caudata*	Two (Rf_1, Rf_2) from *T. timopheevii*	Commercially not used.
Triticum durum	*Aegilops ovata*		Commercially not used
Gossypium hirsutum	*Gossypium anomalum* *Gossypium arboreum*		Commercially not used
Sorghum bicolor	Milo (*S. bicolor*)	One (*Msc*)	Commercially used
Pennisetum americanum	Tifton (*P. americanum*)* Ludhiana (*P. americanum*)*		Commercially used
Helianthus anuus	*H. annuus**	Two (Rf_1, Rf_2)	Commercially used
Oryza sativa	Wild rice (WA type) *O. sativa F. spontanea* Chinsurah Boro II (BT type) Gambiaca (Gam type) O-Shan-Tao-Bai	Eporophytic action Gametophytic action	Widely used used used used
Brassica juncea	*B. napus*	Two genes, cumulative effect	proposed to be used

* Mutant cytoplasm.

** Restoration of fertility by Rf_2 and Rf_4 shows sporophytic control, that is, fertility of pollen grains depends on the genotype of the plant, while that by Rf_3 shows gametophytic control, *i.e.*, fertility of pollen grains depends on their own genotypes.

Utilization in Plant Breeding. Cytoplasmic male sterility may be utilized for producing hybrid seed in certain ornamental species, or in species where a vegetative part is of economic value. But in those crop plants where seed is the economic part, it is of no use because the hybrid progeny would be male sterile.

Cytoplasmic-Genetic Male Sterility

This is a case of cytoplasmic male sterility where a nuclear gene for restoring fertility in the male sterile line is known. The fertility restorer gene, *R*, is dominant and is found in certain strains of the species, or may be transferred from a related species, *e.g.*, in wheat (Table 3.2). This gene restores male fertility in the male sterile line, hence it is known as *restorer gene* (Fig. 3.9). The cases of cytoplasmic male sterility would be included in the cytoplasmic-genetic system as and when restorer genes for them would be discovered. It is likely that a restorer gene would be found for all the cases of cytoplasmic male sterility if a thorough search were made. This system is known in maize, jowar, bajra, sunflower, rice and wheat (Table 3.2).

The plants would be male sterile in the presence of male sterile cytoplasm if the nuclear genotype were *rr*, but would be male fertile if the nucleus were *Rr* or *RR* (Fig. 3.9). New male sterile lines may be developed following the same procedure as in the case of cytoplasmic system (Fig. 3.8). But the nuclear genotype of the pollinator strain used in the transfer must be *rr*, otherwise the fertility would be restored. The development of new restorer strains is some what indirect (Fig. 3.10). First a restorer strain (say R) is crossed with a male sterile line (A). The resulting male fertile plants are used as the female parent in repeated backcrosses with the strain (C), used as the recurrent parent, to which the transfer of restorer gene is desired. In each generation, male sterile plants are discarded, and the male fertile plants are used as females for backcrossing to the strain C. This acts as a selection device for the restorer gene *R* during the backcross programme. At the end of the backcross programme, a restorer line isogenic to the strain C would be recovered.

Utilization in Plant Breeding. The cytoplasmic-genetic male sterility is used commercially to produce hybird seed in maize, bajra and jowar. A generalised scheme for producing a double cross in maize is presented in Fig. 3.11. Alternatively, two fertility restoring inbreds may be used to produce the single cross II. One of the two inbreds, in this case, will have to be detasselled for use as the female parent. Thus all plants in the double cross will be male fertile, since the single cross II will be homozygous for the restorer gene *R*. For producing single crosses, the scheme for production of the single cross II as presented in Fig. 3.11 is followed. A triple cross may be produced by crossing single cross I (Fig. 3.11) with a fertility restoring inbred so that all the plants in the triple cross would be male fertile.

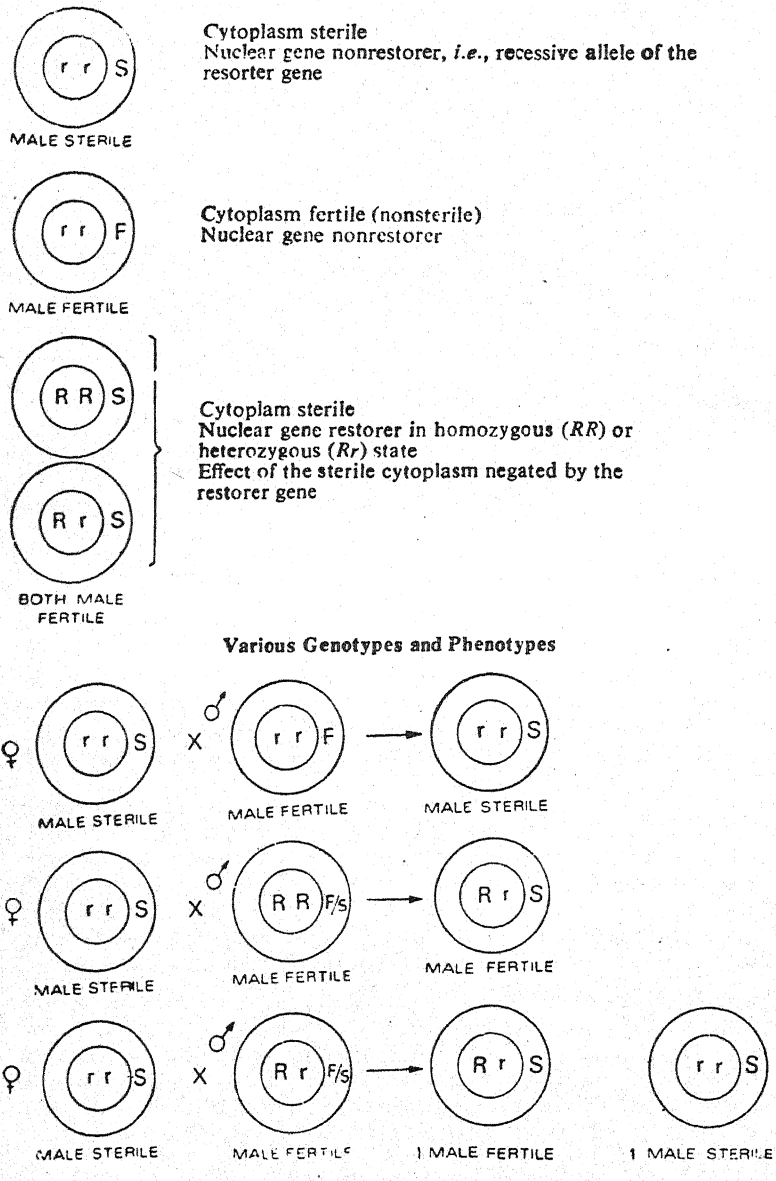
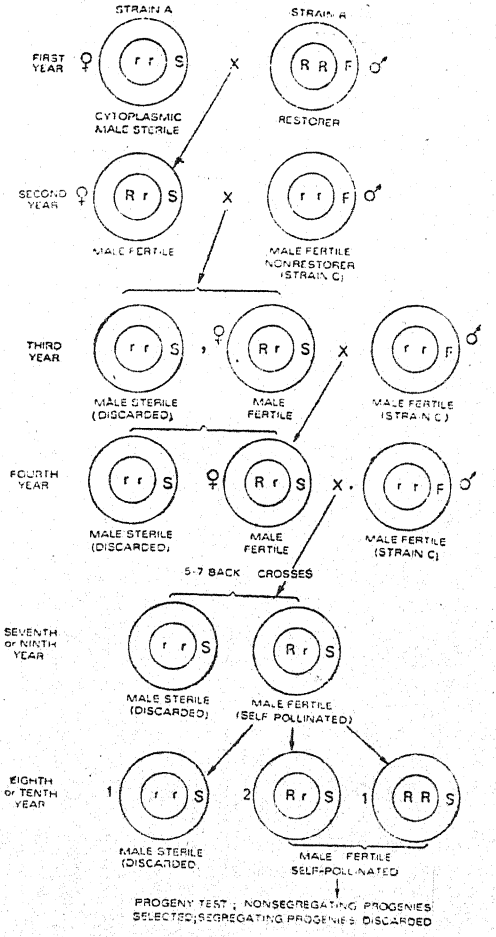


Fig. 3.9. Cytoplasmic-genetic male sterility. Male sterility is due to a sterile cytoplasm ; fertility is restored by a restorer gene, which is usually a dominant nuclear gene. F/S in the cytoplasm indicates that the cytoplasm may be either fertile (F) or sterile (S).



Restorer line crossed to a cytoplasmic male sterile line. This allows selection for *R* gene in the segregating generations.

The resulting male fertile F_1 is crossed to the strain C to which *R* is to be transferred The F_1 is used as female to retain the male sterile cytoplasm.

Male fertile progeny is back-crossed to strain C. Strain C is used as the recurrent male parent.

Male fertile progeny is back-crossed to strain C. Strain C is used as male.

Male fertile progeny self-pollinated

Male fertile progeny selfed. Individual plant progenies grown in the next generation and nonsegregating progenies selected.

Fig. 3 10. Transfer of restorer gene R from a restorer strain (strain R) to a new strain (strain C).

Origin of Male Sterile Cytoplasm. Male sterile cytoplasm arises spontaneously in nature or may be produced by the breeder. The various sources of the male sterile cytoplasm are given below.

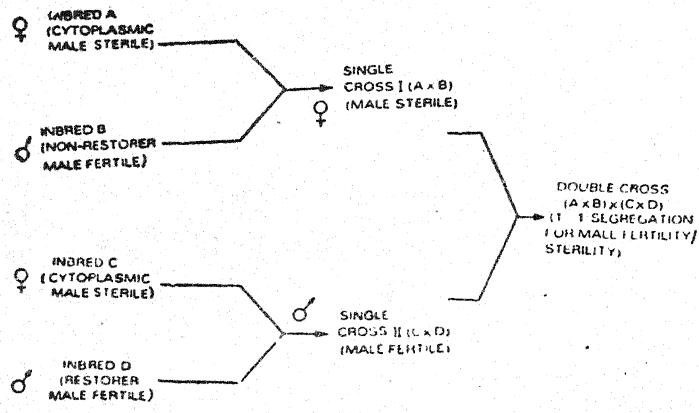


Fig. 3.11. **A generalised scheme for the production of a double cross in maize using cytoplasmic-genetic male sterility.**

1. *Spontaneous Mutation.* Mutant male sterile cytoplasms arise spontaneously in low frequencies. Mutant cytoplasms have been isolated in maize, bajra and sunflower (Table 2.2). It is likely that an extensive search would lead to the isolation of male sterile cytoplasms in almost every crop species.

2. *Interspecific Hybridization.* Transfer of the full somatic chromosome complement of a crop species, through repeated backcrossing, into the cytoplasm of a related wild species often leads to cytoplasmic male sterility. Male sterile cytoplams of wheat have been derived from *Triticum timopheevii* or *Aegilops caudata.* Other examples are listed in Table 2.2. In cross-pollinated crop species, the male sterile cytoplasms have generally originated through mutation, while in self-pollinated crops they have been transferred from related species.

3. *Induction through Ethidium Bromide.* Ethidium bromide is a potent mutagen for cytoplasmic genes or plasmagenes. Male sterile cytoplasm may be induced by seed treatment with ethidium bromide. Such mutants have been induced in some plant species, *e.g.*, *Petunia.*

Limitations of Cytoplasmic-Genetic Male Sterility for Use in Plant Breeding. There are several problems in the utilization of cytoplasmic-genetic male sterility in hybrid seed production in many species. These problems are listed below.

1. *Undesirable Effects of the Cytoplasm.* Male sterile cytoplasms generally have undersirable side effects (Table 2.2). For example, the Texas cytoplasm (cms-T) in maize, by far the most successful cytoplasm commercially, slightly retards growth, yield (2.4%),

plant neight and leaf number ; induces earlier silking and delayed pollen shedding ; and makes the plants highly susceptible to *Helminthosporium* leaf blight. This susceptibility is due to the extreme sensitivity of mitochondria from cms-T genotypes to a toxin produced by the fungus. A good cytoplasm without any side effects is indeed rare. The male-sterile cytoplasms in tobacco could not be used due to their severe undersirable side effects. Restorer genes only restore male fertility ; they are unable to remove the side effects of the male sterile cytoplasms.

2. *Unsatisfactory Fertility Restoration.* In many cases, restoration of fertility is not satisfactory. As a result, these sources cannot be used in the production of hybrid seed.

3. *Unsatisfactory Pollination.* Natural pollination is often not satisfactory, except in wind-pollinated crops like maize. This reduces the production of hybrid seed, and thereby increases its cost. In some species, *e.g.*, *Capsicum*, this has prevented the use of male sterility in hybrid seed production. Poor pollination would always be a major problem in self-pollinators, *e.g.*, wheat. In rice, this problem is overcome by regular tripping of ears in the morning, generally using a rope.

4. Modifier genes may reduce the effectiveness of cytoplasmic male sterility. During backcrosses, while transferring the male sterile cytoplasms, the nuclear genetic background may also be disturbed. This may lead to some pollen production by the male sterile lines.

5. Sometimes, cytoplasm may also be contributed by the sperm which, in the long run, may lead to a breakdown of the male sterility mechanism.

6. Male sterility mechanisms may break down partially under certain environmental conditions resulting in some pollen production by the male sterile lines. This problem has been encountered in maize, bajra and jowar.

7. In crops like wheat, polyploid nature of the crop and undesirable linkages with the restorer gene make it very difficult to develop a suitable restorer (R) line.

SUMMARY

Crop plants reproduce asexually or sexually. Some crops show both sexual and asexual reproduction. Sexually reproducing crops are either self-pollinated or cross-pollinated. Self-pollination is ensured by cleistogamy, opening of flowers after pollination, clustering of anthers over stigma etc. Cross-pollination is promoted by monoecy, dioecy, dichogamy, self-incompatibility or a combination of these mechanisms. Asexually reproducing and cross-pollinated crops are highly heterozygous and generally exhibit strong inbreeding depression. The self-pollinated species are homozygous and do not show inbreeding depression. The different breeding methods are primarily based on the response of crop species to inbreeding. Thus in self-pollinated species a variety consists of homozygous plants, while in cross-pollinated species heterozygosity has to be retained or restored. Thus the mode of reproduction

of crop plants determines their genetic constitution, their response to inbreeding, ease in pollination control and the stability of varieties after release.

Self-incompatibility may be heteromorphic or homomorphic. Heteromorphic system shows sporophytic control of the pollen incompatibility reaction. But the homomorphic system exhibits both gametophytic and sporophytic control. Male sterility is either genetic, cytoplasmic or cytoplasmic-genetic. Both incompatibility and male sterility are useful in the production of hybrid seed. The cytoplasmic-genetic system has been the most commonly used in hybrid seed production.

QUESTIONS

1. What do you understand from mode of reproduction ? Discuss its relevance to plant breeding programmes with the help of suitable examples.
2. Briefly describe the various modes of reproduction prevalent in crop plants.
3. What are the differences between the following : (i) Parthenogenesis and apomixis, (ii) Gametogenesis and sporogenesis, (iii) Self-incompatibility and male sterility, and (iv) Often cross-pollinated and cross-pollinated species.
4. Define sporogenesis. Briefly describe the process of micro- and mega-sporogenesis with the help of suitable diagrams.
5. Describe in brief the process of gametogenesis in plants.
6. Write short notes on the following : (i) Apomixis, (ii) Parthengenesis, (iii) Self-incompatibility, (iv) Male sterility, (v) Triple fusion, (vi) Heterostyly, (vii) Anthesis, (viii) Often cross-pollinated species, (ix) Cleistogamy, (x) Restorer gene, (xi) Chasmogamy, and (xii) Dichogamy.
7. Describe the various mechanisms which promote cross-pollination (or ensure self-pollination) in plants.
8. How would you determine the mode of reproduction of a species ? Briefly describe the procedure for determining the amount of cross-pollination in a species.
9. Give a suitable classification for self-incompatibility. Describe the different types of self-incompatibility with the help of suitable examples.
10. What is male sterility ? List the various types of male sterility found in plants. Explain the cytoplasmic-genetic male sterility with the help of suitable examples and diagrams.
11. Discuss the relevance of self-incompatibility and male sterility in plant breeding. Briefly explain the limitations of each system.
12. In the field, a single plant of a crop species does not set seeds/pods. How would you decide whether the lack of seed set in this plant is due to male sterility, self-incompatibility or some other reason ?
13. How would you maintain the following ? (i) A self-incompatible line, (ii) A genetic male sterile line, (iii) A cytoplasmic male sterile line, (iv) A cytoplasmic-genetic male sterile line, and (v) A restorer line.
14. Define self-, cross- and often cross-pollination. Discuss their effects on the genetic composition of populations and their relevance to crop improvement.

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CHAPTER 4

Qualitative and Quantitative Characters

The two basic requirements of plant breeding are variation and selection. *Variation in a character is a must for an improvement in that character.* If all the plants in a population were identical for a character, no improvement in that character would be possible. In such a case variation must be created through hybridization, mutation or polyploidy (see later). Selection is the second basic step in crop improvement. *Selection involves the identification and isolation of desirable plants.* The progeny from selected plants may be released as a variety if they are found suitable and superior to the existing varieties.

The selection of plants from a population is almost always based on their appearance, *i.e., phenotype.* Phenotype has both heritable and nonheritable components. The *heritable* component is due to the genes present in plants, that is, *genotype.* The *nonheritable* component consists of the effects of environment. The value of progeny obtained from a selected plant, therefore, would largely depend upon the relative contributions by the heritable and nonheritable components to its phenotype. Clearly, the breeder should be thoroughly familar with the laws of inheritance and the relative importance of the genotype and the environment in determining the concerned phenotype.

In the foregoing chapters, and indeed throughout this text, it has been assumed that the reader is familiar with the elementary laws of genetics. However, the laws of genetics that concern us the most are briefly reviewed here. *Phenotype* is the appearance of a plant with respect to a particular character, such as plant height, flower colour etc., or it may refer to a group of characters. The development of a character is mainly governed by one or a group of genes. But the genes do not produce the characters directly. The genes produce different proteins, which often act as enzymes. These enzymes catalyse specific biochemical reactions. Thus the primary function of a gene is to produce a specific enzyme (more precisely, a polypeptide) which catalyses a specific biochemical reaction.

These biochemical reactions finally lead to the development of various characters of the plant, that is, the phenotype. The relationship between the primary (enzyme production) and the ultimate (phenotype production) functions of a gene is well understood for a limited number of characters, while for most of the characters it is not known. The genes and the characters they produce are often separated by several biochemical and physical events. Thus character development is likely to be modified by the action of other genes in the genotype of a plant, and also by the environment. However, the degree to which the gene function is affected by these factors varies considerably from one character to the other.

Some characters are little affected by other genes, *i.e.*, the genetic background, or the environment. Such characters are generally governed by one or few genes with large, easily detectable effects. These genes are known as *oligogenes*. The characters produced by oligogenes show distinct classes and are known as *qualitative characters*. On the other hand, the development of many characters is very much affected by the genetic background and, more particularly, by the environment. These characters are governed by several genes with small individual effects ; these genes are known as *polygenes*. The characters produced by polygenes are referred to as *quantitative characters*, because they do not show clear-cut classes and have to be studied by measurement. Inheritance of both qualitative and quantitative characters follows the laws of Mendel. But the effects of individual genes in the two cases are totally different. Consequently, the techniques used to study the two types of characters are also different. These differences stem primarily from the ease in classifying plants into distinct phenotypic classes in one case and the inability to do so in the other. In crop improvement, both the types of characters are important. Many characters of economic value show the qualitative, while several others exhibit the quantitative mode of inheritance.

INHERITANCE OF QUALITATIVE CHARACTERS

Mendel proposed the laws of inheritance based on his studies with qualitative characters. Subsequently, a large amount of additional information has been collected to supplement the laws of Mendel. It is universally accepted that the characters are determined by genes, and that genes are normally located in chromosomes which are present in the nucleus of cell. In body cells or somatic cells of higher plants, each chromosome has an identical partner or *homologue*. During meiosis, the two homologous chromosomes pair and move to the opposite poles at the first anaphase. The gametes produced, therefore, receive only one of the two homologues of each chromosome. Fertilization restores the somatic chromosome number in zygotes since a zygote is produced by fusion of two gametes, one male and the other female.

Apparently, in somatic cells each gene has two copies present in the two homologues. The two copies may be identical or they

may be different. The behaviour of genes during gametogenesis and fertilization is identical with that of the chromosomes. Each character has two, sometimes more, alternative forms known as *contrasting characters*. For example, seed shape in peas (*Pisum sativum*) may be round or wrinkled. The contrasting characters are determined by alternative forms of the same gene which are referred to as *alleles*. When two alleles are brought together through an appropriate cross, generally one of the alleles expresses itself, while the other is unable to do so. The allele that expresses itself in the presence of another allele is *dominant*, and the one that is unable to express itself is *recessive*. For example, the allele for round seed shape (*W*) may be brought together with that for wrinkled seed shape (*w*) by crossing a plant producing round seeds with one having wrinkled seeds. The seeds resulting from this cross (F_1) are round. Thus allele *W* expresses itself at the expense of the allele *w*. Therefore, *W* is *dominant* and *w* is *recessive*.

At the time of gamete formation, the two alleles present in the F_1 (Ww) *separate and pass into different gametes ; this is known as segregation.* Segregation of the two alleles of one gene produces two types of gametes, *e.g.*, *W* and *w*, in equal frequencies. Random union among these gametes produces three different types of zygotes

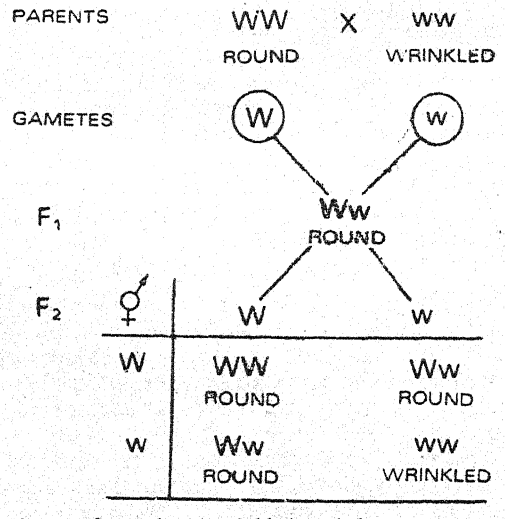


Fig. 4.1. Inheritance of round and wrinkled seeds in pea.

Table 4.1. Frequency of different genotypes and phenotypes produced by segregation of a single gene *Ww* (round Vs. wrinkled seeds) in peas.

Genotype	*Frequency*	*Phenotype*	*Frequency*
WW	1	round	3
Ww	2	round	
ww	1	wrinkled	1

(*WW*, *Ww* and *ww*) which produce two phenotypes (round and wrinkled) in the ratio 3 : 1 (Fig. 4.1, Table 4.1). When two or more genes are segregating together, often their segregation is independent of each other, *i.e.*, at the time of gamete formation an allele of one gene goes with each of the two alleles of the other gene or genes with equal frequency. In case of two genes, independent segregation produces four different types of gametes in equal frequencies. Random union among these gametes produces nine genotypes which give rise to four phenotypes in the ratio 9 : 3 : 3 : 1 (Fig. 4.2, Table 4.2).

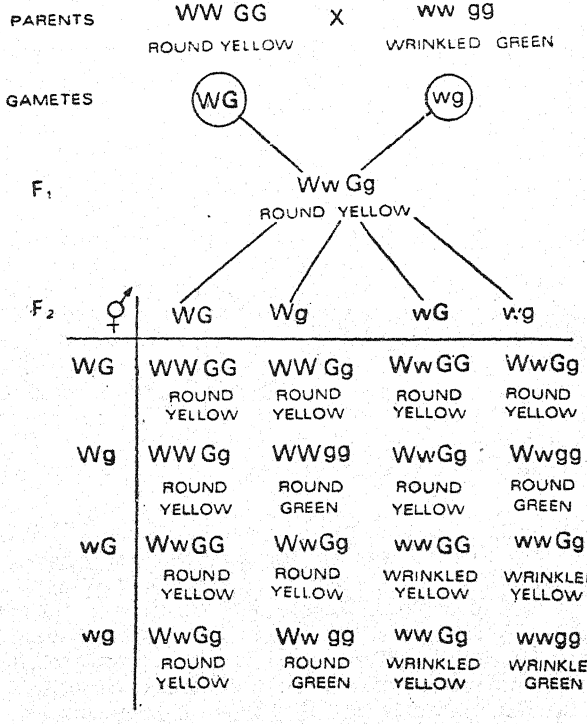


Fig. 4.2. Independent segregation of seed shape (round/wrinkled) and seed colour (yellow/green) in pea.

When the two copies of a gene (alleles) present in an individual are identical, *e.g.*, *WW* or *ww*, it is known as a *homozygote*. But when two alleles are dissimilar, *e.g.*, *Ww*, it is termed as a *heterozygote*. Both homozygous and heterozygous conditions produce the same phenotype, *e.g.*, round seed, when there is full dominance. Thus the same phenotype may be produced by different genotypes. The homozygous (*WW*) and heterozygous (*Ww*) round-seeded plants cannot be separated on the basis of their phenotypes. But they can be easily differentiated by studying their progeny, *i.e.*, from their breeding

Table 4.2. Genotypic and phenotypic frequencies produced by independent segregation of two nonalleles *Ww* and *Gg* (round *vs.* wrinkled and yellow *vs.* green seeds) in pea.

Genotype	*Frequency*	*Phenotype*	*Frequency*
WWGG	1	round yellow	9
WWGg	2	round yellow	
WwGG	2	round yellow	
WwGg	4	round yellow	
WWgg	1	round green	3
Wwgg	2	round green	
wwGG	1	wrinkled yellow	3
wwGg	2	wrinkled yellow	
wwgg	1	wrinkled green	1

behaviour. The determination of genotype or genotypic value of a plant by studying the progeny produced by it is known as *progeny test.* Progeny test is an essential part of all modern plant breeding programmes.

PLEIOTROPY

Generally, each oligogene is supposed to govern a single character. But there are several instances where a single major gene (oligogene) is known to affect more than one character. *The phenomenon of a single major gene affecting more than one character is known as pleiotropy,* and such a gene action is known as *pleiotropic gene action.* For example, a case of pleiotropy has been reported in wheat for a gene producing awned spikes. It was found that addition of the awned character to an awnless variety Onas, by recurrent backcrossing, increased the yield as well as seed size. Removal of the awns from the awned variety Baart had a comparable but the opposite effect.

Clearcut cases of pleiotropy are but limited. This is mainly due to the difficulty in demonstrating the pleiotropic effects of a gene. To investigate pleiotropy, two lines must be developed which are identical with respect to all other genes except for the gene under investigation. Such lines are known as *isogenic lines.* Isogenic lines may be developed in two ways. In the *first method,* F_1 and the successive generations from a suitable cross are selfed. In each generation, heterozygotes for the gene in question are selected. After 8-10 generations of selfing, such plants may be expected to be homozygous for all the genes except for the gene in question. The two homozygotes isolated from the progeny of a single heterozygous plant at this stage may be expected to differ only for the gene under study. The *second method* of producing isogenic lines consists of recurrent back-crossing for 10 or more generations. Thus in a study of pleiotropy, about 10 generations are required to produce isogenic lines, obviously a time consuming process. Isogenic lines have been extensively used to investigate pleiotropic effects of many genes in a number of crop species.

Pleiotropy may result from true pleiotropic gene action or it may be due to a closely-linked gene or a group of genes. The linkage may often be sufficiently close to keep the genes together during the production of isogenic lines. Therefore, even the study of isogenic lines does not establish pleiotropy beyond doubt. An example of this situation is furnished by the rough and smooth awns in barley (*Hordeum vulgare*). Semismooth-awned derivatives from the rough-awned Atlas barley out-yielded Atlas as well as the rough-awned derivatives. The semismooth and rough-awned derivatives were obtained by 10 backcrosses to Atlas. But a careful study later revealed that the higher yielding ability of the semismooth-awned derivatives was due to linked genes and not due to pleiotropy.

In crop improvement, it makes little difference whether the favourable side effects of a gene are due to pleiotropy or due to tight linkage. But when the side effects are unfavourable, true pleiotropy offers no chance of removing these side effects. But in the case of tight linkage, the side effects may be removed by raising sufficiently large populations to recover the recombinants lacking these side effects.

PENETRANCE AND EXPRESSIVITY

Generally, oligogenes express themselves in all the individuals that carry them, and their expression is fairly uniform. But some oligogenes fail to express themselves in some of the individuals carrying them. Such genes are said to have *incomplete penetrance. Thus penetrance is the ability of a gene to express itself in the individuals carrying it in the appropriate genotype.* For a dominant gene, the appropriate genotype will be the dominant homozygote as well as the heterozygote, while for a recessive gene it will be the recessive homozygote. For example, there is a gene that causes partial chlorophyll deficiency in the cotyledonary leaves of Lima beans (*Phaseolus lunatus*). But only about 10 per cent of the seedlings carrying this gene show chlorophyll deficiency. This gene, therefore, has a penetrance of 10 per cent, *i.e.*, it expresses itself in 10 per cent of the individuals carrying it. On the other hand, the gene which expresses itself in every individual that carries it is said to have a *complete penetrance.*

Some oligogenes, in contrast, show variable degrees of expression in different individuals having the appropriate genotype for this gene. Such genes are said to have *variable expressivity. Expressivity, therefore, denotes the ability of a gene to express itself uniformly in all the individuals that carry it.* A gene that expresses itself uniformly in all the individuals has *uniform expressivity*, while those that are unable to do so have *variable expressivity.* The gene producing chlorophyll deficiency in the Lima bean (*P. lunatus*) seedlings exhibits variable expressivity in addition to incomplete penetrance. In some seedlings, the cotyledonary leaves have no chloro-

phyll, in the others the chlorophyll is absent from the leaf tip, while in still others only the margins of leaves are affected. Thus a single gene with variable expressivity may produce a number of phenotypes as if more than one gene were involved. Almost always, genes showing incomplete penetrance also show variable expressivity. In fact, incomplete penetrance is the consequence of variable expressivity when the phenotype produced in some of the individuals is so small as to be undetectable. Incomplete penetrance and variable expressivity are the consequences of the effects of environmental factors on the expression of concerned genes.

Incomplete penetrance and variable expressivity confuse the relationship between genotype and phenotype. Consequently, such genes pose difficulty in selection of desirable types. In such cases, progeny tests for more than one generation may be required to establish the genotype of the plants beyond a reasonable doubt.

THRESHOLD CHARACTERS

Some genes require a specific environment, *e.g.*, a particular temperature, for their expression. *Characters whose development depends upon a specific environment are known as threshold characters.* For example, a mutant gene in barley (*Hordeum vulgare*) produces albino seedlings at temperatures below 8°C. But when the seedlings carrying this gene are grown at 19°C or above, they develop into normal green seedlings. Incomplete penetrance of some genes may be due to a threshold requirement.

The knowledge of threshold may be helpful in the identification of desirable genotypes. For example, disease resistant and disease susceptible plants cannot be differentiated unless the plants are exposed to the concerned disease. Similarly, lodging resistant and lodging susceptible plants can be separated only when conditions that favour lodging are present. Many other similar instances may be encountered in crop improvement.

MODIFYING GENES

There are many genes that do not seem to have any effect of their own, but they increase or decrease the expression of other oligogenes. *These genes are known as modifying genes since they modify the effects of other genes.* Individual modifying genes have small effects which are cumulative in nature. Consequently, their effects are quantitative in nature. Modifying genes are generally studied in the same manner as quantitative characters. It is likely that most of the major genes are affected by a group of modifying genes. For example, spotting in mice is affected by modifying genes and has been thoroughly studied. A dominant gene *S* is necessary for spotted colour. But the effect of *S* is modified by several modifying genes that affect the degree of spotting. The modifying genes produce almost a continuous series from uniform-black to uniform white. Thus the action of a major gene may be considerably affected by the genetic background in which it is present.

Similarly, the degree of self-incompatibility produced by different *S* alleles is considerably affected by the genetic background (modifying genes). Many disease resistance genes are also known to be affected by the genetic background.

In crop improvement, while transferring a major gene, the breeder should be careful to retain, eliminate or accumulate modifying genes according to the needs of the situation. It has been suggested that the crop improvement may soon reach a stage when breeders will have accumulated all the available major genes. Further improvement would then largely depend on the accumulation of favourable modifying genes.

GENE INTERACTION

Many, perhaps most, qualitative characters are determined by two or more oligogenes. *These genes show various relationships with each other in producing the character. These relationships are known as gene interaction.* In Japanese varieties or rice (*O sativa*), two dominant genes, *Rc* and *Rd*, are needed to produce red pericarp. When one of the two genes or both the genes are in the recessive state, the pericarp is white. This is known as *comlementary gene action*, and shows a 9 : 7 ratio in F_2. In maize (*Zea mays*), a gene *Pr* has no effect of its own. But *Pr* modifies the action of *R*, which produces red colour. In the presence of *R*, *Pr* changes the red colour to purple. This type of gene interaction is referred to as *supplementary gene action*; it produces a 9 : 3 : 4 ratio in F_2. *Inhibitory gene action* is characterised by an inhibitory gene which stops the action of another gene, and has no effect of its own. Red colour production in maize is stopped by an inhibitory gene *I*; the recessive allele *i* is not inhibitory. The F_2 ratio in this case is 13 : 3.

Another gene interaction, often known as *epistatic gene action*, produces 12 : 3 : 1 ratio in F_2. In barley (*Hordeum vulgare*), a dominant gene *Y* produces yellow seed coat, while another gene *B* produces black seed coat. When genes *B* and *Y* are together, the effect of *Y* cannot be seen due to the masking effect of *B*, *i.e.*, black colour. It is more appropriate to denote this gene action as *masking gene action* since epistasis is generally used to denote interaction between two nonallellic genes, irrespective of the manner in which they interact.

Some characters are governed by two genes that produce identical effects, whether together or alone. The alternative form of the chraracter results only when both the genes are in the recessive form. The ratio in F_2 is 15 : 1, and the interaction is known as *duplicate gene action.* For example, the nonfloating habit in rice is governed by two genes, Dw_1 and Dw_2, either alone or together. The floating habit results only when both the genes are in the recessive form, *i.e.*, $dw_1\ dw_1\ dw_2\ dw_2$. Some genes show *polymeric*

gene action. In this case, the two genes produce identical effects individually, but their effect is increased when both the genes are present together. In barley, gene *A* or *B* alone produces awns of medium length. But when *A* and *B* are present together, long awns result. The double recessive condition, *aa bb*, produces awnless lemma. The ratio in F_2 is 9 : 6 : 1.

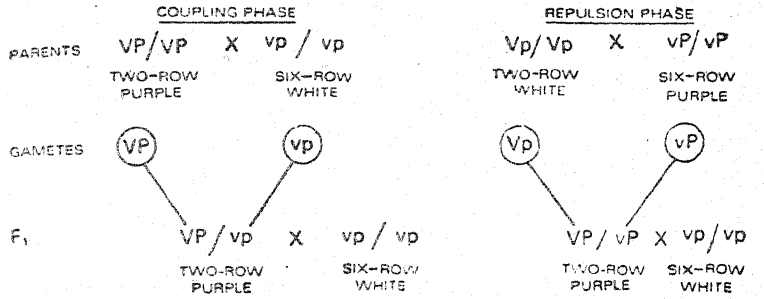
Often more than two genes may by involved in an interaction, and these genes may have different relationships with each other. This leads to much more complex F_2 ratios. However, if one understands the various interactions between two genes as described above, one could work out the various ratios that may be expected in such complex interactions.

The chemical basis of gene interaction has been worked out in several cases. Gene interactions arise because each character is generally produced by a series of biochemical reactions. In this chain of biochemical reactions, each reaction provides the substrate for the next reaction. Each reaction is usually controlled by one gene. Each gene generally produces an enzyme which catalyses a biochemical reaction. A simple gene interaction situation may be as follows. If a gene controlling a reaction is in recessive state, it would not produce an active enzyme ; hence the reaction controlled by this gene would not take place. The reactions occurring beyond this one in the reaction chain would also not occur due to a lack of substrates even though the genes governing them were present in the respective dominant form. This interaction would produce complementary gene action. For example, HCN (hydrocyanic acid) production in clovers (*Trifolium sp.*) is governed by two dominant genes, say, *A* and *B*. Gene *A* controls a reaction that produces a cyanogenic glycoside. The enzyme produced by the gene *B* releases HCN from this glycoside. Thus HCN production would require the presence of both the genes *A* and *B* in the dominant state. If the gene *A* were in recessive state, the cyanogenic glycoside would not be produced, and there would be no substrate for the enzyme produced by gene *B*. And if the gene *B* were in recessive state, HCN would not be released from the cyanogenic glycoside produced by gene *A*.

LINKAGE

So, far, we have assumed that the genes segregate independently. However, *genes often show a tendency to be inherited together, that is, a tendency to pass to the same gamete during segregation, and do not show independent segregation. This phenomenon is known as linkage.* The genes that show linkage are situated in the same chromosome. Each chromosome is transmitted intact as a unit during meiosis. Consequently, the genes situated in a chromosome are also transmitted together. But during meiosis, there is exchange of chromatin material between homologous chromosomes ; this is known as *crossing over.* Crossing over, therefore, leads to

recombination between linked genes. The frequency of recombination between any two linked genes depends upon the distance between them. *Thus the chief effect of linkage is to reduce the frequency of recombination between linked genes* (Fig. 4 3, Table 4.3).



Test-cross progeny

♂ +	*vp*	*Phenotype*	*Frequency (%)* *Coupling*	*Repulsion*
VP	*VP/vp*	Two-row, purple	40.3	9.7
vp	*vp/vp*	Six-row, white	40.3	9.7
Vp	*Vp/vp*	Two-row white	9.7	40.3
vP	*vP/vp*	Six-row, purple	9.7	40.3

*In barley, six-row condition of spikes is recessive to two-row condition, and white lemma colour is recessive to purple lemma colour.

Fig. 4.3. Inheritance of number of rows in the spike and lemma colour in barley.

Linkage may be in *coupling phase* in which two dominant genes are linked together, *e.g.*, *VP/vp*, or in *repulsion phase* where one dominant and one recessive gene are linked, *e.g.*, *Vp/vP*. The coupling or repulsion phases alter drastically the frequencies of various phenotypes in F_2 and other segregating generations but have no effect on the frequency of recombination (Fig. 4.3, Table 4.3).

Liakage is of considerable value in plant breeding. *When two favourable genes are linked, they tend to be transmitted together, and are easily combined in the progeny. In case of a tight linkage crossing over less than one per cent), selection for only one of the characters would be necessary.* This may be an advantage in cases where selection for one of the characters is easier than that for the other. For example, resistance for stem rust and loose smut are closely linked in barley (*H. vulgare*). The techniques of inoculation for stem rust are much easier than those for loose smut. Therefore, in a breeding programme, the breeder may select for stem rust resistance only. The loose smut resistance would be selected for due to its close linkage with stem rust resistance. *But a linkage between a desirable and an undesirable gene causes delay in crop improvement programmes. The breeder would have to grow a sufficiently large* F_2

Table 4.3. Effect of linkage in coupling and repulsion phases on the recovery of *AB/AB* genotype from F_2.

*Cross-over value (p)**	*Frequency of AB/AB individuals in F_2 (in percent)*	
	Coupling (AB/ab)	*Repulsion (aB/Ab)*
0·50 (independent segregation)	6.25	6.25
0·40	9.00	4.00
0.30	12.25	2.25
0.25	14.06	1.56
0 20	16.00	1 00
0.10	20.25	0.25
0.05	22 56	0 0625
0.02	24.01	0.01
0.01	24.50	0·0025
p	$100\times[\frac{1}{4}(1-p)^2]$	$100\times(\frac{1}{4}P^2)$

* *p* represents the proportion of cross-over gametes produced by the F_1 (double heterozygote *AB/ab* or *Ab/aB*). This proportion is conveniently determined by studying testcross ($F_1\times ab/ab$) progeny. The proportion of recombinant types in the testcross progeny is *p*. In coupling phase, the recombinant types would be *Ab* and *aB*, while in the repulsion phase they would be *AB* and *ab*.

population to allow the recovery of desirable recombinants. The size of required F_2 would depend upon the closeness of linkage ; the closer the linkage, the larger the size of F_2. Certain wheat (*T. aestivum*) strains, such as, CI 12633, have a gene for stem rust resistance from *Triticum timopheevii*. This gene is closely linked with late maturity. This linkage was broken by growing a large population to recover early rust resistant recombinants.

QUANTITATIVE CHARACTERS

Quantitative characters are governed by several genes ; each gene has small effect which is usually cumulative. These characters are considerably affected by the environment. Quantitative characters show a continuous variation and it is not possible to classify them into distinct classes. The examples of such characters are yield, plant height, days to flower, days to maturity, protein content, seed size, etc. If a large number of observations on a quantitative character are plotted in a frequency distribution curve, often a bell-shaped normal curve would be obtained. Since classfication of individuals into distinct classes is not possible, inheritance of quantitative characters cannot be studied through classical techniques of genetic analysis applied so successfully to the study of qualitative characters. Therefore, inheritance studies on quantitative characters have to employ statistical procedures. A special branch of mathematics, *Biometry*, has developed as a result of the construction of special models and procedures to deal with the various aspects and problems of quantitative inheritance.

THE MULTIPLE FACTOR HYPOTHESIS

The inheritance of quantitative characters was a matter of great controversy for a considerable time. Francis Galton and his followers considered that the Mendel's laws of inheritance could not explain the continuous variation of such characters. Others, notably Hugo de Vries, regarded continuous variation to be nonheritable. In 1906, Yule suggested that many genes with small, similar effects could produce continuous variation. He proposed that these genes were transmitted according to the laws of Mendel.

In 1908, Nilsson-Ehle presented experimental evidence to support the hypothesis of Yule. He studied the inheritance of seed

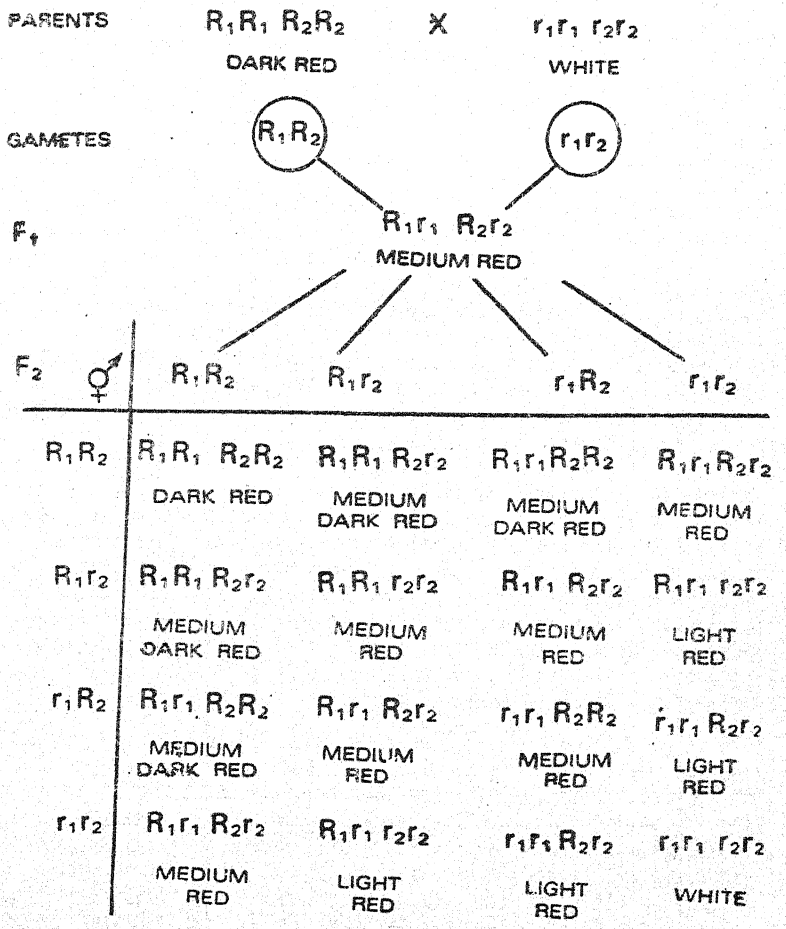


Fig. 4.4. Inheritance of seed colour in wheat

Table 4.4. Genotype and phenotype frequencies produced by the segregation of two genes with cumulative effect, $R_1r_1\ R_2r_2$, for seed colour in wheat.

Genotype	*Frequency*	*Number of dominant alleles*	*Phenotype*	*Frequency*
$R_1R_1\ R_2R_2$	1	4	Dark red	1
$R_1r_1\ R_2R_2$	2	3	Medium-dark red	4
$R_1R_1\ R_2r_2$	2	3	Medium-darkred	
$R_1r_1\ R_2r_2$	4	2	Medium red	6
$R_1R_1\ r_2r_2$	1	2	Medium red	
$r_1r_1\ R_2R_2$	1	2	Medium red	
$R_1r_1\ r_2r_2$	2	1	Light red	4
$r_1r_1\ R_2r_2$	2	1	Light red	
$r_1r_1\ r_2r_2$	1	0	White	1

colour in wheat (*Triticum sp.*) and oats (*Avena sp.*). The F_2 generations from various crosses had red and white grains in the ratios of 3 : 1, 15 : 1 or 63 : 1. It is clear that the seed colour in these crosses was governed by one, two and three genes, respectively. For further analysis, let us consider the cross giving 15 red : 1 white seeds. A closer study of seed colour revealed that the seeds classified as red differed in the intensity of colour. The red seeds could be grouped into four distinct classes : dark red, medium-dark red, medium red and light red. These four classes were in the ratio 1 : 4 : 6 : 4. Thus the 15 : 1 ratio was actually 1 : 4 : 6 : 4 : 1. It can be seen from Fig. 4.4 and Table 4.4 that *this ratio is obtained if it is assumed that the seed colour is governed by two genes with similar, small and additive effects.* The dominant alleles of the two genes produce red colour, while the recessive alleles produce no colour. The intensity of colour depends on the number of dominant alleles present. Thus genotypes $R_1R_1\ r_2r_2$, $r_1r_1\ R_2R_2$ and $R_1r_1\ R_2r_2$ will produce the same intensity of colour since they all have two dominant alleles. Thus Nilsson-Ehle was able to show that *certain characters are governed by genes that have small and cumulative effect.* This, in essence, is the *multiple factor hypothesis* or *pologenic inheritance.*

The data of Nilsson-Ehle demonstrated that genes possessed the necessary qualities, *i.e.*, small and cumulative effect, to explain continuous variation. But the evidence was not conclusive because the character he studied exhibited discontinuous variation. East in 1916 presented conclusive evidence that quantitative characters are governed by many genes with small and cumulative effect. He studied the inheritance of corolla length in *Nicotiana longiflora.* The two parents had contrasting corolla lengths, *viz.*, 40 and 93 mm, and the F_1 was intermediate with 63 mm long corolla. The F_2 showed a continuous variation for corolla length which could not

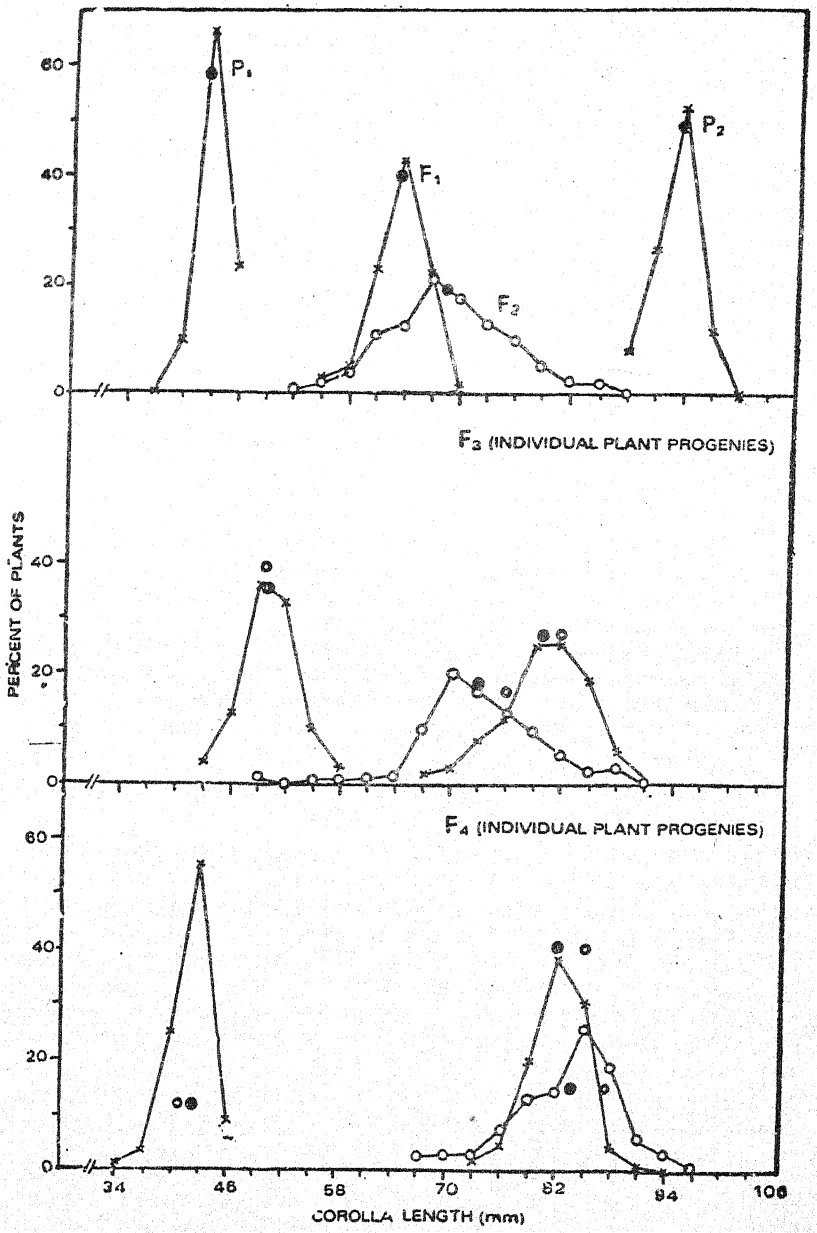


Fig. 4.5. Frequency distribution of the two parents, F_1, F_2, F_3 and F_4 progenies for corolla length in *Nicotiana longiflora*. The closed circles represent the mean of the various populations. In the cases of F_3 and F_4 progenies, the open circles alongside the closed ones denote the corolla length of the parent plant of the previous generation from which the progeny was derived.

be grouped into separate classes. To analyse his data, East used statistical parameters, such as, frequency distribution, mean and coefficient of variation. The essential features of his study are summarised below and presented in Fig. 4.5.

1. The two parents were inbreds and almost homozygous. But there was variation within the two parents for corolla length. This variation, therefore, may be assumed to arise from environmental effects.

2. The F_1 mean was intermediate between those of the two parents. This would be expected if multiple factors with cumulative effect governed this trait.

3. The variability in F_1 was comparable to that in the parents. Again, all the F_1 plants would have the same genotype. Therefore, the variability present in F_1 was obviously produced by the environment.

4. The F_2 generation was much more variable than the parents and the F_1. East postulated that the greater variability in F_2 resulted from the segregation and recombination of Mendelian genes.

Thus it is clear that these observations correspond closely to the expectations based on Mendelian inheritance. It is not possible to determine the number of genes controlling the character. But as few as 5 genes with cumulative gene action would produce a continuous curve in F_2. Although with 5 genes there would be only 11 classes, each class separated by 4·5 mm, the identity of these classes would disappear due to the effects of environment as is evident from the data on the parents and F_1.

Confirmatory evidence for Mendelian inheritance of corolla length may be obtained from F_3, F_4 and F_5 generations. Based on Mendelian principles, it may be predicted that,

1. F_2 plants with markedly different corolla lengths should produce F_3 progenies that differ from each other.
2. Different F_2 progenies would show different variabilities. Some F_3 progenies should be as variable as the F_2, while others would be less variable. Variability in some F_3 progenies may be comparable to that in the parents. These expectations arise due to the differences in the genotypes of different F_2 plants ; some are comparable to the F_1, while some are more like the parents (Fig. 4.4).
3. In F_4 and the succeeding generations, variability of a progeny would be equal to or less than that of the progeny from which it arose, but never more. This is because a plant present in a F_3 progeny cannot be more heterozygous than the F_2 plant which produced that progeny since selfing causes a decrease in heterozygosity. The same applies to the succeeding generations.
4. Since F_3 and later generations are produced by selfing, it may be expected that homozygosity would increase in every succeeding generation. Therefore, the variability in individual plant pro-

genies should also decrease with the advancing generation, *i.e.*, in terms of variability $F_2 > F_3 > F_4 > F_5$ etc.

The data from F_2, F_4 and F_5 generations agreed closely with the above four predictions. The F_3 families differed significantly for mean corolla length. The mean corolla length of F_3 families was associated with the corolla length of the F_2 plants from which they were derived. The variability pattern in F_2, F_4 and F_5 also followed the predicted pattern. These observations tend to confirm that the corolla length is governed by several genes with small and cumulative effect. It may be pointed out that polygenes (multiple factors) do not always have additive gene action. They generally show dominance and epistatic actions as well.

POLYGENIC INHERITANCE AND CONTINUOUS VARIATION

The chief difference between qualitative and quantative traits lies in the degree to which they are affected by the environment. Qualitative characters are little or not at all affected by the environment, while quantitative characters are considerably affected. The chief effect of environment is to mask the differences between different genotypes and to produce a continuous variation in the character. It can be shown that a character governed by a single gene with additive effect would show discrete distribution when the genotype alone determines the phenotype, *i.e.*, there is no effect of the environment on the phenotype. As the magnitude of the effect of environment on the phenotype increases, the phenotypic classes progressively overlap each other and form a continuous curve. The distribution becomes roughly similar to the normal curve when the contribution of environment is 50 per cent. If the contribution of environment increases to 75 per cent, the distribution tends to approach normal distribution. Experimental evidence suggests that in case of most of the quantitative characters, the contribution of environment varies from 10-50 per cent, while in some cases, such as yield, it may often be much higher. *Thus environmental effects would produce a continuous variation even if the number of genes governing a character was very small, or even one.*

A similar continuous variation will be produced if the number of genes governing the character is increased, and it is assumed that the effect of environment is zero. As the number of genes is increased, the number of phenotypic classes also increases. If one assumes 12 pairs of genes, there would be 25 phenotypic classes, and the distribution would tend to become continuous. With cumulative gene effect, the distribution would be symmetrical, *i.e.*, similar to the normal distribution. Even with complete dominance, the distribution would be close to the normal one. In practice, the effect of environment would never be zero. Therefore, the distribution will be a continuous one even with complete dominance.

The multiple factor hypothesis or polygenic inheritance would produce continuous distribution, more specifically normal distribu-

tion, provided the number of genes governing the character is large and the character is affected by environment. This conclusion would be correct even in the case of complete dominance. The polygenic inheritance of quantitative characters is universally accepted. But it is now recognised that the alleles of polygenes may show dominance in addition to the additive effect, and nonalleles may show interallelic interactions of different types. A series of mathematical models has been developed to estimate the importance of allelic and nonallelic interactions in quantitative inheritance.

ROLE OF ENVIRONMENT IN QUANTITATIVE INHERITANCE

Quantitative characters, by definition, are considerably affected by environment. The main result of this effect is that the relationship between genotype and phenotype is partially or completely hidden, *i.e.*, the phenotype does not reveal the genotype. For example, if the environmental effect is zero, the phenotype would be produced by the genotype only. Thus there will be complete correspondence between phenotype and genotype. However, the effect of environment is seldom zero. As a result, the phenotype is the result of a joint action of genotype and environment. Obviously, the effect of environment is not heritable and it cannot be passed on to the progeny. Thus only that part of phenotype that is the result of genotype is heritable.

In crop improvement, the breeder selects plants on the basis of their phenotype. The effectiveness of selection would largely depend on the proportion of phenotype due to the genotype. Therefore, it is important for the breeder to know the extent to which environment influences different quantitative characters. The estimate of the effect of environment on a character may be obtained from a simple experiment. The experiment consists of a large number of genotypes, *i.e.*, strains, varieties etc., grown in a replicated trial. The number of genotypes should be as large as possible, but it should not be less than 30. The data thus obtained are subjected to analysis of variance appropriate for the experimental design used. The analysis of variance based on a Randomised Block Design is given in Table 4 5.

Table 4.5. Analysis of variance for genotypes grown in a replicated trial according to a Randomised Block Design.

Source of variation	*d. f.* (*degrees of freedom*)	*Expectation of MS* (*mean squares*)
Genotypes	$G-1$	$\sigma^2e+R\sigma^2g$
Replications	$R-1$	$\sigma^2e+G\sigma^2r$
Error	$(R-1)(G-1)$	σ^2e
Total	$(RG)-1$	

Where, G and R are the numbers of genotypes and replications, respectively ; and σ^2e, σ^2r and σ^2g denote the variances due to composite error, replication and genotype, respectively.

The phenotype may be described according to a mathematical model to facilitate statistical analysis and interpretation. The phenotypic mean, *i.e.*, $\overline{X}$ of a given genotype from the trial may be expressed as,

$$\overline{X}=\mu+g+e+ge$$

where, μ is the general population mean, that is the mean phenotype of all possible genotypes grown under all possible environments ; g is the effect of genotype ; e is the effect of environment ; and ge denotes the interaction between genotype and environment. The genotype × environment interaction signifies that the relative performance of various genotypes is affected by the environment. In other words, the relative performance of the genotypes would change if the environment is changed. For example, genotype A may be superior to genotype B in one environment, but in another environment it would be inferior to B. However, if genotype × environment interaction is absent, genotype A will be superior (or inferior) to B in all the environments.

Using the mean squares (variances) from an analysis of variance table (Table 4.5), an estimate of the contribution of genotype to the phenotype may be obtained. The mean square due to genotypes has an expectation of $\sigma^2e+R\sigma^2g$. The σ^2e represents the remainder mean square or a composite error term, which includes differences in plot effects within a replication, sampling error, and errors in measurement. The σ^2g, on the other hand, represents the component of variance due to genetic differences among the genotypes. Thus the genetic component of variance may be estimated as follows.

Genotypic variance (σ^2g)=(MS due to genotypes − MS due to error)/R.

Where, R is the number of replications.

$$=[(\sigma^2e+R\sigma^2g)-\sigma^2e]/R$$
$$=R\sigma^2g/R$$
$$=\sigma^2g$$

Similarly, phenotypic variance (σ^2p) can be estimated by totalling the genotypic and environmental variances.

$$\sigma^2p=\sigma^2g+\sigma^2e$$

Table 4.6. Analysis of variance for nitrogen content per plant in mung *V.(radiata)* grown on agar slants in symbiosis with MO5 cowpea *Rhizobium*. Since the agar slants do not contain combined nitrogen, the nitrogen content of the plant is a reliable measure of its nitrogen fixing ability in symbiosis.

Source of variation	*d.f.*	*Sum of squares (SS)*	*Mean squares (MS)*
Genotypes	48	519.419	10.821
Replications	2	0.082	0.041
Error	96	12.294	0.128

Since, in practice, the *MS* due to genotypes and error are based on a sample, it is more appropriate to use the symbols V_g and V_p in place of σ^2_g and σ^2_p. The method may be illustrated by using the analysis of variance data presented in Table 4.6. Table 4.6 is based on data on total nitrogen content per plant in mung genotypes planted in a Randomised Block Design with three replictations.

V_g=(*MS* due to genotypes—*MS* due to error)/R
=(10.821 − 0.128)/3
=10.693/3 or 3.564

Similarly,

$V_p = V_g + V_e$ (V_e is the *MS* due to error)
=3.564+0.128
=3.692

An alternative method of estimating the genotypic variance consists of growing the two parents (P_1 and P_2), the F_1 and the F_2 in a trial. Variances are estimated for P_1, P_2, F_1 and F_2 separately. In the case of P_1, P_2 and F_1, the variances are due to environment only since all the plants within each of them have the same genotype. The variance for F_2, on the other hand, has both genetic and environmental components. The environmental variance (V_e) may be estimated as follows.

$$V_e=(V_{P_1}+V_{P_2}+V_{F_1})/3$$

and the variance for F_2 may be written as,

$$V_{F_2}=V_g+V_e$$

Therefore, $V_g=V_{F2}-[(V_{F1}+V_{P_2}+V_{F_1})/3]$

This is illustrated by using data on days to flowering in case of bajra (*Pennisetum americanum*).

V_{F_2}=23.02 ; V_{P_1}=10.19 ; V_{P_2}=9.29 ; and V_{F_1}=9.46

Therefore, V_g=23.02−[(10.19+9.29+9.46)/3]
=23.02−9.65
=13.37

It is apparent that the effect of a genotype can be estimated only in relation to other genotypes, *i.e.*, in relation to μ. Therefore, the performance of genotypes should be evaluated under a number of environments to obtain reliable estimates of genotypic and phenotyic variances. The most accurate estimates would be obtained if all possible genotypes were tested under all possible environments. But in practice, this is simply not possible. The experimenter usually attempts to investigate the effects of relatively more important environmental factors, such as, temperature, rainfall, soil fertility etc. Generally, these factors are considered to be represented by locations and years, and the breeder does not study the effects of individual environmental factors. This is done because of the near impossibility of manipulating and controlling the various environmental factors over the experimental area. If desired, the breeder may evaluate the effects of easily manageable factors, such as, irrigation water, fertilizer application, population density, date of

planting, etc. However, generally the experiment is conducted at two or more locations in two or more years. In case of such an experiment, the mean phenotype is given by,

$$\overline{X} = \mu + g + r + l + y + gl + gy + ly + gly + e$$

where r, l and y denote the effects of replication, location and years, respectively, and e is the composite error term. Interaction between genotype and locations is represented by gl, between genotype and years by gy, between locations and years by ly, and among genotypes and locations and years by gly.

Therefore, when genotypic variance is estimated from a trial conducted at only one location in one year, it also contains the interaction variances, *i.e.*, it is the sum of $\sigma^2 g$, $\sigma^2 gl$, $\sigma^2 gy$ and $\sigma^2 gly$. Therefore, the genotypic variance estimated from a single trial is biased upward. The interaction variances ($\sigma^2 gl$, $\sigma^2 gy$, $\sigma^2 gly$) can be estimated from trials coducted at several locations during several seasons. The genotypic variances estimated from such trials would be unbiased and raliable.

COMPONENTS OF GENETIC VARIANCE

The genetic variance estimated from trials using homozygous lines arises from differences between homozygotes. But the breeder is mostly concerned with segregating populations, *e.g.*, F_2, F_3 etc., derived from crosses. In such cases, the genotypes used in the study are not homozygous, but they will be homozygous for some genes and hetrozygous for the others. In case of only additive gene action, it does not matter whether a gene is homozygous or heterozygous and with what other genes it is present. But strictly additive gene action is an oversimplification of the real situation ; the polygenes show dominance as well as epistatic gene actions.

Fisher in 1918 divided the genotypic variance into three components : (1) additive, (2) dominance and (3) interaction components.

Additive Component. It is the component arising from differences between the two homozygotes for a gene, *e.g.*, *AA* and *aa*.

Dominance Component. Dominance component is due to the deviation of the hetrozygote (*Aa*) from the averages of the two homozygotes (*AA* and *aa*). This is sometimes referred to as *intra-allelic interaction.*

Interaction or Epistatic Component. The epistatic component results from interaction between two or more genes.

Later, Hayman and Mather partitioned the *epistatic component* into three types of interactions, *viz.*, additive × additive, additive × dominance and dominance × dominance. The various components of gene action, their notations and descriptions are summarised in Table 4.7.

The primary objective of *biometrical genetics*, a branch of genetics concerned with the application of biometry to genetic

studies, is to identify and estimate the various components of genetic variance. Several techniques are available for this purpose, but each of them suffers from one limitation or the other (see Chapter 5).

Table 4.7. Different types of gene actions involving two genes *Aa*, *Bb*.

Component of genetic variation	*Specific Symbol*	*Description*	*General Symbol*
Additive	*da*	The difference between *AA* and *aa*	*d*
	db	The difference between *BB* and *bb*	
Dominance	*ha*	The deviation of *Aa* from the average of *AA* and *aa*	*h*
	hb	The deviation of *Bb* from the average of *BB* and *bb*	
Epistasis	*da*×*db*	Additive×additive effect due to interaction between *AA* and *BB*	*i*
	da×*hb* and *ha*×*db*	Additive×dominance interaction due to interaction between *AA* and *Bb*, and *Aa* and *BB*, respectively	*j*
	ha×*hb*	Dominance×dominance interaction due to interaction between *Aa* and *Bb*	*l*

ESTIMATION OF COMPONENTS OF GENETIC VARIANCE

The additive and dominance components of genotypic variance may be estimated from a simple experiment. The experiment consists of two parents, P_1 and P_2, their F_1 and F_2, and the two backcrosses, B_1 ($F_1 \times P_1$) and B_2 ($F_1 \times P_2$). The variances from these generations are used to obtain estimates of additive and dominance variances. These estimates are based on the assumption that epistasis is absent. The environmental component of the phenotypic variance is estimated by averaging the variances of P_1, P_2 and F_1, the nonsegregating generations.

The theoretical basis for estimating the additive and dominance components of variance may be explained by taking an example of a single gene *A* with two alleles *A* and *a*. Three genotypes are possible for this gene which may be represented as follows (Fig 4.6).

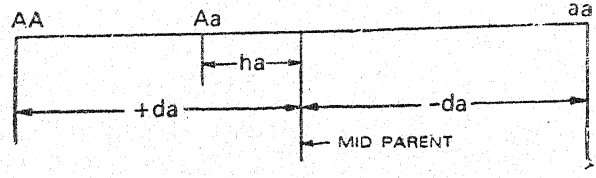


Fig. 4.6. Relationship between homozygotes and heterozygotes at a single locus (midparent is the average of *AA* and *aa*).

The symbol da denotes the additive effect of the positive allele A, while $-da$ is the additive effect of the negative allele a. The positive and negative signs denote the direction of effects of the two alleles on the phenotype. Since there will be more than one gene affecting a character, the phenotype of a homozygous line would be,

$$X=\Sigma(+d)+\Sigma(-d)+c$$

where, $\Sigma+d$ is the additive effect of positive alleles at all the loci affecting the character, $\Sigma-d$ is the additive effect of all the negative alleles, and c denotes the effects of the genotypic background and the environment. Thus the phenotype of a pureline would be the sum total of positive and negative additive effects of all the genes affecting the character, and the value c. For a single locus, if $h=0$ (there is no dominance), the F_1 will be intermediate between the two homozygotes. But quantitative characters are governed by more than one gene. Therefore, if F_1 does not deviate from the mid-parent value (that is, average phenotype of the two parents) it does not necessarily mean that dominance is absent. It may simply mean that dominance effects of different genes have opposite signs and thus cancel each other out, *i.e.*, $\Sigma h=0$. When all the positive alleles are present in one strain and all the negative alleles are present in the other, the two parents would differ by $2\Sigma d$, because one parent will have the phenotype $\Sigma d+c$ and the other will have the phenotype $\Sigma-d+c$. But in practice, both strains would contain some positive and some negative alleles. Consequently, the difference between them would be always less than $2\Sigma d$.

On the basis of above considerations, we may estimate the variances of different segregating genetations with respect to a single gene, Aa, as follows :

Generation, F_2 :	Genotype ,	AA	Aa	aa
	Phenotype ,	da	ha	$-da$
	Frequency ,	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$

Thus, Mean $=\frac{1}{4}da+\frac{1}{2}ha-\frac{1}{4}da$ (since the population size is 1

$=\frac{1}{2}ha$

Sum of squares $=\frac{1}{4}(da)^2+\frac{1}{2}(ha)^2+\frac{1}{4}(-da)^2$

And sum of squares of deviations

from F_2 mean $=[\frac{1}{4}(da)^2+\frac{1}{2}(ha)^2+\frac{1}{4}(-da)^2]-(\frac{1}{2}ha)^2$

$=(\frac{1}{2}da^2+\frac{1}{2}ha^2)-\frac{1}{4}ha^2$

$=\frac{1}{2}da^2+\frac{1}{4}ha^2$

Since we are dealing with a population of size 1, the sum of squares is also the variance of F_2. Since there will be more than one gene affecting the character, the formula may be rewritten as follows.

$$V_{F_2}(\text{Variance of } F_2)=\tfrac{1}{2}\Sigma d^2+\tfrac{1}{4}\Sigma h^2$$

For simplicity, we may replace Σd^2 with D, and Σh^2 with H. Since there will be an environmental component, E, we may add E to the equation to complete the formula for V_{F_2}.

$$V_{F_2}=\tfrac{1}{2}D+\tfrac{1}{4}H+E$$

Similarly, for the backcrosses,

Generation, B_1 : Genotype , AA Aa
Phenotype , da ha
Frequency , $\frac{1}{2}$ $\frac{1}{2}$

Thus, mean $= \frac{1}{2}da + \frac{1}{2}ha$

Sum of squares $= \frac{1}{2}(da)^2 + \frac{1}{2}(ha)^2$

and, sum of squares of deviations

from B_1 mean $= [\frac{1}{2}(da)^2 + \frac{1}{2}(ha)^2] - (\frac{1}{2}da + \frac{1}{2}ha)^2$
$= (\frac{1}{2}da^2 + \frac{1}{2}ha^2) - (\frac{1}{4}da^2 + \frac{1}{4}ha^2 + \frac{1}{2}da\ ha)$
$= \frac{1}{4}da^2 + \frac{1}{4}ha^2 - \frac{1}{2}da\ ha$

The above is also V_{B_1} since the size of population is 1. Summation over all the loci and addition of the environmental effect will give,

$$V_{B_1} = \tfrac{1}{4}\Sigma d^2 + \tfrac{1}{4}\Sigma h^2 - \tfrac{1}{2}\Sigma dh + E$$
$$= \tfrac{1}{4}D + \tfrac{1}{4}H - \tfrac{1}{2}\Sigma dh + E$$

Similarly, for the other backcross,

Generation, B_2 : Genotype : Aa aa
Phenotype : ha $-da$
Frequency : $\frac{1}{2}$ $\frac{1}{2}$

Thus, mean $= \frac{1}{2}ha + \frac{1}{2}(-da)$
$= \frac{1}{2}ha - \frac{1}{2}da$

Sum of squares $= \frac{1}{2}(ha)^2 + \frac{1}{2}(-da)^2$

and, sum of squares of deviations

from B_2 mean $= [\frac{1}{2}(ha)^2 + \frac{1}{2}(-da)^2] - (\frac{1}{2}ha - \frac{1}{2}da)^2$
$= [\frac{1}{2}ha^2 + \frac{1}{2}da^2) - (\frac{1}{4}ha^2 + \frac{1}{4}da^2 - \frac{1}{2}da\ ha)$
$= \frac{1}{4}ha^2 + \frac{1}{4}da^2 + \frac{1}{2}da\ ha$

This is also V_{B_2}, as in the case of V_{B_1}. Summation over all the loci and addition of the environmental component would give,

$$V_{B_2} = \tfrac{1}{4}\Sigma h^2 + \tfrac{1}{4}\Sigma d^2 + \tfrac{1}{2}\Sigma dh + E$$
$$= \tfrac{1}{4}H + \tfrac{1}{4}D + \tfrac{1}{2}\Sigma dh + E$$

When we add the variances for B_1 and B_2 generations, we get,

$$V_{B_1} + V_{B_2} = (\tfrac{1}{4}D + \tfrac{1}{4}H - \tfrac{1}{2}\Sigma dh + E) + (\tfrac{1}{4}D + \tfrac{1}{4}H + \tfrac{1}{2}\Sigma dh + E)$$
$$= \tfrac{1}{2}D + \tfrac{1}{2}H + 2E$$

Thus the variances of the different segregating generations may be summarised as follows.

$$V_{F_2} = \tfrac{1}{2}D + \tfrac{1}{4}H + E$$
$$V_{B_1} + V_{B_2} = \tfrac{1}{2}D + \tfrac{1}{2}H + 2E$$

From these equations, we can estimate D as follows.

$$2V_{F_2} = D + \tfrac{1}{2}H + 2E$$
$$-(V_{B_1} + V_{B_2}) = \tfrac{1}{2}D + \tfrac{1}{2}H + 2E$$
$$2V_{F_2} - V_{B_1} + V_{B_2} = \tfrac{1}{2}D$$

An estimate of the environmental variances can be obtained by averaging the variances of the nonsegregating generations.

$$E=(V_{P_1}+V_{P_2}+V_{F_1})/3$$

We can now estimate H by subtracting the values of $\frac{1}{2}D$ and E from V_{F_2}.

$$V_{F_2}-\tfrac{1}{2}D-E=(\tfrac{1}{2}D+\tfrac{1}{4}H+E)-\tfrac{1}{2}D-E$$
$$=\tfrac{1}{4}H$$

Estimation of D and H is illustrated using data on days to flower in bajra (*P. americanum*).

$V_{P_1}=10.19$ $V_{F_2}=23.02$
$V_{P_2}=9.29$ $V_{B_1}=17.68$
$V_{F_1}=9.48$ $V_{B_2}=19.30$

The estimate of D is obtained as follows.

$$2V_{F_2}=46.04$$
$$V_{B1}+V_{B_2}=36.98$$
Thus, $\frac{1}{2}D=2V_{F_2}-(V_{B_1}+V_{B_2})$
$=46.04-36.98$
$=9.06$

Therefore, $D=18.12$

The estimate of E is given by,

$$E=(V_{P_1}+V_{P_2}+V_{F_1})/3$$
$=(10.19+9.29+9.48)/3$
$=9.65$

Finally, $\frac{1}{4}H=V_{F_2}-\frac{1}{2}D-E$
$=23.02-9.06-9.65$
$=4.31$

Therefore, $H=17.24$

HERITABILITY

In crop improvement, only the genetic component of variation is important since only this component is transmitted to the next generation. *The ratio of genetic variance to the total variance, i.e., phenotypic variance, is known as heritability.* Thus heritability denotes the proportion of phenotypic variance that is due to genotype, *i.e.*, heritable. Heritability may be represented as follows.

Heritability $(H)=V_g/V_p$
or $=V_g/(V_g+V_e)$

where, V_g, V_p and V_e are the genetic, phenotypic and environmental components of variances, respectively.

Heritability can be estimated by three different methods listed below.

1. From analysis of variance table of a trial consisting of a large number of genotypes.

2. Estimation of V_g and V_e from the variances of F_2, P_1, P_2 and F_1 generations.
3. Parent-offspring regression upon doubling provides an estimate of heritability. Thus $H=2b$, where b is the regression of progeny means on parental values.

The use of analysis of variance data for estimating heritability is explained using the data from Table 4.6. It has been shown that the estimate of Vg in this case is 3.564 and that of Ve is 0.128. The heritability, therefore, will be,

$$H=V_g/(V_g+V_e)$$
$$=3.564/(3.564+0.128) \text{ or } 3.564/3.692$$
$$=0.914$$

When expressed in per cent, $H=91.4$ percent.

The *second* method of estimation of heritability consists of the estimation of V_g and V_e from variances of F_2, P_1, P_2 and F_1 as explained previously. The heritability is then estimated using the formula $H=V_g/(V_g+V_e)$.

Heritability estimated from the above three methods is known as *broad sense heritability*. Broad sense heritability estimates are valid when homozygous lines are studied. However, when we are dealing with segregating generations, the genetic variance consists of additive and dominance components (assuming there is no epistasis). Since in self-pollinated crops we develop homozygous lines, the dominance component will not contribute to the phenotype of the homozygous lines derived from the population. Consequently, in such cases, only the additive component (D) of the genetic variance is relevant. Therefore, the ratio of additive component of variance to the total phenotypic variance is a more appropriate estimate of heritability, and it is referred to as *narrow sense heritability*.

For the estimation of narrow sense heritability, the components of genetic variance are estimated by the procedure described earlier in this chapter, or by some other suitable technique. The appropriate value of D is used to determine heritability.

$V_{F_2}=\frac{1}{2}D+\frac{1}{4}H+E$ (V_{F_2} is the phenotypic variance of F_2)

The value of D is estimated as described before. The heritability is calculated as follows :

$$H(ns)=\tfrac{1}{2}D/V_{F_2}$$
$$=9.05/23.02$$
$$=0.394 \text{ or } 39.4 \text{ per cent}$$

Only $\frac{1}{2}D$ is used for the estimation of heritability since the phenotypic variance of F_2 has only $\frac{1}{2}D$ as its component.

Estimates of heritability serve as a useful guide to the breeder. The breeder is able to appreciate the proportion of variation that is due to genotypic (broad sense H) or additive (narrow sense H) effects, that is, the heritable portion of variation in the first case.

and the portion of genetic variation that is fixable in purelines in the latter case. If heritability of a character is very high, *e.g.*, 0.8 or more, selection for such a character should be fairly easy. This is because there would be a close correspondence between genotype and phenotype due to a relatively smaller contribution of environment to the phenotype. But for a character with low heritability, say less than 0.4, selection may be considerably difficult or virtually impractical due to the masking effect of environment on the genotypic effects.

SUMMARY

The various characteristics of plants may be qualitative or quantitative in nature. *Qualitative characters* show distinct classes, are little affected by the environment (both external and internal), and are governed by one or few genes with large distinct effects, *i.e.*, *oligogenes*. The *quantitative traits*, on the other hand, show continuous distribution, are generally influenced by the environment, and are controlled by several genes with small and cumulative effect, *i.e.*, *polygenes*. Classical genetic analysis is based on study of qualitative traits. Mendel showed that when two alleles are brought together in the hybrid, they segregate at the time of gamete formation. Usually, nonalleles (two different genes) segregate independently, but often they may be linked, *i.e.*, they may show a tendency to be inherited together. Linkage reduces the recovery of recombinant genotypes. Thus a linkage between two desirable genes is an advantage in plant breeding. But linkage between one desirable and one undesirable gene presents problems in crop improvement.

Oligogenes generally govern a single trait, but sometimes they may affect several unrelated traits; this is known as *pleiotropy*. Clearcut cases of pleiotropy are limited; many cases may be due to linked genes. Some genes are unable to express themselves in all the individuals that carry them (*incomplete penetrance*), while others show variable expression (*variable expressivity*). Certain genes require a specific environment for their expression; such characters are known as *threshold characters*. Such genes present problems in selection. Action of many oligogenes may be affected by *modifying genes*, which change the intensity of expression. Modifying genes have no known action of their own, except the modification of the action of the major gene. Many qualitative traits are controlled by two or mpre oligogenes which interact in various ways. The various interactions produce different ratios in F_2, *e g*, 9 : 7, 9 : 3 : 4, 13 : 3, 12 : 3 : 1, 15 : 1 and 9 : 6 : 1. Often more than two genes may be involved leading to a complex relationship among the genes.

In 1908, Nilsson-Ehle put forward the *multiple factor* hypothesis to explain the inheritance of seed colour in wheat. According to this hypothesis, some characters are governed by several genes with small and cumulative effect. This would produce a graded variation which would tend to become continuous due to environmental influences. East, in 1916, presented conclusive evidence that quantitative characters are governed by multiple factors or *polygenes*. This view is universally accepted, but it is recognised that the polygenes may show dominant and epistatic gene actions in addition to cumulative gene effects.

The phenotype of quantitative traits is considerably influenced by environment; it has environment and genotype × environment interaction components. Variance due to environment may be estimated from a replicated trial consisting of several genotypes. But the interaction component may be estimated only when the trial is conducted in more than one environment, preferably at different locations and during different years. From such a trial, the *genetic* and *phenotypic variances* can be computed, which may be used to

estimate heritability. *Heritability*, $Vg/(Vg+Ve)$, denotes the proportion of phenotypic variance that is due to genetic reasons.

The genetic variance may be further subdivided into variances due to *additive*, *dominance* and *epistatic* gene actions. These components of genetic variance can be estimated by using an appropriate biometrical analysis. An estimate of variances due to additive and dominance gene effects can be obtained from a trial consisting of two purelines (parents), their F_1, F_2 and the two backcrosses. In such an analysis, epistasis is assumed to be absent. From these estimates, *narrow sense heritability* is estimated, which is the ratio of additive variance to the total phenotypic variance. The narrow sense heritability denotes the proportion of phenotypic variance that is fixable under selection.

QUESTIONS

1. Differentiate between the following : (i) Pleiotropy and linkage, (ii) Penetrance and expressivity, (iii) Isogenic lines and purelines, (iv) Dominance and epistasis, and (v) Modifying genes and polygenes.
2. Define the following : Phenotype, segregation, gene interaction, oligogenes, polygenes, qualitative characters and quantitative characters.
3. Explain how the effectiveness of selection is affected by : (i) Heritability, (ii) Pleiotropy, (iii) Penetrance, (iv) Expressivity, (v) Linkage, (vi) Threshold characters and (vii) Epistasis.
4. Briefly describe the contributions by the following scientists : Mendel, Francis Galton, Hugo de Vries, Yule, Nilsson-Ehle, East, Fisher, Hayman and Mather.
5. Briefly describe the multiple factor hypothesis or polygenic inheritance. How did the findings of Nilsson-Ehle show that quantitative characters are inherited according to the Mendelian laws ?
6. 'Environment plays an important role in the expression of metrical traits'. Discuss with the help of suitable examples.
7. What are the various components of genetic variance ? How would you estimate the values of D and H components from the variances of different generations ?
8. Define heritability and briefly describe the methods for its estimation. How does a knowledge of heritability aid selection ? Is selection possible when the heritability for a trait is 100 per cent or zero per cent.

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CHAPTER 5

Biometrical Techniques in Plant Breeding

Biometry or *biometrics* is the science that deals with the application of statistical procedures to the study of biological problems. Similarly, *biometrical genetics* is that branch of genetics which attempts to unvavel the inheritance of quantitative traits using statistical concepts and procedures; it is also known as quantitative genetics for obvious reasons. The various statistical procedures employed in biometrical genetics are called *biometrical techniques.* Several books and a huge literature are available on the concepts and the procedures of biometrical techniques. The present discussion is limited in scope to a brief description of some common biometrical techniques and their relevance to plant breeding. It is intended to outline the type of genetic information obtainable from each biometrical technique, and the manner in which that information is helpful in plant breeding programmes.

Biometrical techniques are useful to the plant breeders in four different ways : (1) in the assessment of genetic variability present in a population (range, variance, standard deviation, coefficient of variation, standard error, D^2 statistic, metroglyph analysis), (2) in the selection of elite genotypes from mixed populations (correlation, path and discriminant function analyses), (3) in the choice of parents and breeding procedures (diallel, partial diallel, line × tester, generation means, triallel, quadriallel, biparental cross and triple test cross analyses), and (4) in determining the varietal adaptation (stability analysis).

ASSESSMENT OF VARIABILITY

An insight into the magnitude of variability present in a crop species is of utmost importance as it provides the basis for effective selection. The total variation (phenotypic variation) present in a population arises due to genotypic and environmental effects. *Phenotypic variability* is the observable variation present in a character in a population ; it includes both genotypic and environmental components of variation and, as a result, its maguitude differs under different environmental conditions. *Genotypic variation,* on the other hand, is the component of variation which is due to the genotypic differences among individuals within a population, and is the main concern of the plant breeder.

Fisher was the first to divide, in 1918, the genetic variance into three components, *i.e.*, additive, dominance and epistatic variances. *Additive variance* refers to that portion of genetic variance which is produced by the deviations due to the average effects of the alleles of the genes at all segregating loci. *Dominance variance* arises due to the deviation from additive scheme of gene action resulting from intraallelic interaction, that is, dominance. And *epistatic variance* arises due to the deviations as a consequence of interallelic interaction.

The epistatic variance was further divided by Hayman and Mather into the following three components : (1) additive × additive, (2) additive × dominance and (3) dominance × dominance interactions (see, Chapter 4, p. 92). Wright suggested the partitioning of genetic variance into two components, *viz.*, additive and nonadditive (dominance and epistatic components), of which only the additive component contributes to genetic advance under selection. Mather divided the phenotypic variance into three components, namely, (1) heritable fixable (additive variance), (2) heritable nonfixable (dominance and epistatic components), and (3) nonheritable nonfixable (environmental fraction). In fact, the heritable fixable component of the phenotypic variance will include the additive × additive fraction of the epistatic variance as well. Further, the total phenotypic variance may be partitioned as (1) fixable (additive and additive × additive components) and (2) non-fixable (dominance, epistatic minus additive × additive and environmental fractions) components.

The above discussion may be summarised as follows :

$\sigma^2P=\sigma^2G+\sigma^2E$;

$\sigma^2G=\sigma^2A+\sigma^2D+\sigma^2I$; and

$\sigma^2I=\sigma^2AA+\sigma^2AD+\sigma^2DD$

where, σ^2P = phenotypic variance, σ^2G = genotypic variance, σ^2A = additive variance, σ^2D = dominance variance, σ^2I = epistatic variance, σ^2AA = additive × additive variance, σ^2AD = additive × dominance variance, and σ^2DD = dominance × dominance variance.

In a random mating population with no epistasis and zero inbreeding, the covariance between a parent and its offspring is, $\frac{1}{2}\sigma^2A$; the covariance among half-sibs is, $\frac{1}{4}\sigma^2A$; and the covariance among full-sibs is $\frac{1}{2}\sigma^2A+\frac{1}{4}\sigma^2D$. These relationships change with the level of inbreeding in the population.

Numerous studies have been conducted to estimate the magnitudes of various components of the genetic variance. The following general conclusions may be drawn from these studies. *Genetic variability for important agronomic traits in almost all the crops is mainly due to the additive genetic variance. The non-additive variance also exists in nearly all crop species and for many important traits, but it is generally smaller in magnitude than the additive component.*

The variability present in breeding populations can be assessed in the following three ways : (1) using simple measures o

(2) by estimating the various components of variance, and (3) by studying the genetic diversity.

Simple Measures of Variability. They include range, variance, standard deviation (SD), standard error (SE) and coefficient of variation (CV), which may be defined as follows.

1. **Range.** It is the difference between the lowest and the highest values present in the observations included in a sample.
2. **Arithmetic Mean.** It is also known simply as *mean* or average, and is computed by dividing the sum of all the observations in a sample by their number. Thus,

$$\bar{X}=(\Sigma X)/N$$

where, $\bar{X}$ = mean, Σ = summation of, X = any observation in a sample, and N = number of observation in the sample.

3. **Variance.** It is expressed as the sum of squares of the deviations of all observations of a sample from its mean, and divided by the degrees of freedom (= N − 1). It is generally denoted by s^2 or v for the estimates from samples, and as σ^2 for those from populations. It may be represented as follows.

$$s^2=[\Sigma X^2-\{(\Sigma X)^2/N\}]/(N-1)$$

4. **Standard Deviation.** It is the square root of variance, and is designated as s or SD (in case of sample), or as σ (in case of population). Thus,

$$s \text{ or } SD = \sqrt{s^2}$$

5. **Coefficient of Variation.** It is an important measure of variability, and is commonly represented by CV. CV is the per cent ratio of the standard deviation of a sample to its mean. Thus,

$$CV=(SD/\bar{X})\times 100$$

From replicated data, phenotypic, genotypic and environmental coefficients of variation can be estimated.

6. **Standard Error.** It is the measure of the mean difference between sample estimate of mean ($\bar{X}$) the population parameter (μ), *i.e.*, it is the measure of uncontrolled variation present in a sample. It is estimated by dividing the estimate of standard deviation by the square-root of the number of observations in the sample, and is denoted by SE. Thus,

$$SE=SD/\sqrt{N}$$

The magnitude of variation may also be assessed by estimating the components of total variance of a sample. This involves crossing a number of genotypes or strains in a definite fashion and the evaluation of progeny thus obtained in replicated trials. Diallel, partial diallel, line × tester, generation means etc. analyses are used to estimate the components of genetic variance. These techniques will be discussed separately later in this chapter.

The *third approach* for assessing variability involves the study of genetic diversity within a population. Two important biometrical techniques, *viz.*, D^2 statistic and metroglyph analysis, are commonly

used for this purpose. A brief description of these techniques is given below.

D^2 Statistic. The variability among different genotypes of a species is known as *genetic diversity*. Genetic diversity arises either due to geographical separation or due to genetic barriers to crossability. Variability differs from diversity in the sense that the former has observable phenotypic differences, whereas the latter may or may not have such an expression. One of the potent techniques of measuring genetic divergence is the D^2 statistic proposed by Mahalanobis in 1936. This technique measures the forces of differentiation at two levels, namely, intracluster and intercluster levels, and thus helps in the selection of genetically divergent parents for their exploitation in hybridization programmes.

Genetic diversity plays an important role in plant breeding because hybrids between lines of diverse origin generally display a greater heterosis than those between closely related strains. For example in fescue, the magnitude of heterosis increased with the genetic divergence in morphological traits and flowering time, and also with the geographical origin of the parents. The magnitude of heterosis in alfalfa and cotton was positively associated with genetic diversity between the parents involved in the cross. In maize, increased genetic differences between inbred lines results in a greater heterosis in their hybrids. However, the maximum heterosis generally occurs at an optimal or intermediate level of diversity. The D^2 technique has been used in assessing the variability in crops like maize, jowar, bajra, wheat, linseed, cotton, tobacco, alfalfa and brassicas (Moll and Stuber, 1974).

In addition to aiding in the selection of divergent parents for hybridization, D^2 statistic measures the degree of diversification and determines the relative proportion of each component character to the total divergence. The genotypes grouped together are less divergent than the ones which are placed in different clusters. The clusters which are separated by the greatest statistical distance show the maximum divergence. Three important points should be taken into consideration while selecting parents on the basis of D^2 statistic. These points are : (1) the relative contribution of each character to the total divergence, (2) the choice of clusters with the maximum statistical distance, and (3) the selection of one or two genotypes from such clusters. Other characters, like disease resistance, earliness, quality, etc., should also be considered. Crossing of the selected lines in a diallel fashion may generate some useful segregants.

Metroglyph Analysis. This is a semigraphic method of studying variability in a large number of germplasm lines taken at a time. This technique was developed by Anderson in 1957 to investigate the pattern of morphological variation in crop species. The analysis of variation is based on the mean values for different traits. To begin with, two characters exhibiting the highest variability are identified. One of these traits, say, pod number in green gram, is used as the

X-axis, while the other, *e.g.*, seed yield/plant is plotted on the Y-axis. For each line, the mean values of X and Y are used to determine its position, which is marked by a small circle or 'glyph'. Thus each line is represented by a glyph on the graph. For easy identification, exotic lines (lines imported from other countries) may be represented by solid circles (glyphs), while indigenous lines may be designated by open circles (Fig. 5.1). The other characters for the different lines are represented by rays on the respective glyphs (Fig. 5.1). Each

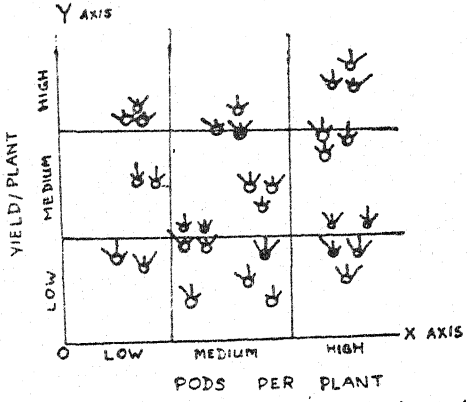


Fig. 5.1. A metroglyph representation of five quantitative traits in 32 lines of green gram. The X-axis depicts pods/plant, while the Y-axis represents yield/plant. Each line is represented by a circle ; the position of a circle on the graph is determined by the mean pods/plant and yield per plant of the concerned line. The three rays emanating from each circle represent the three remaining traits ; the rays are either short, medium or long showing low, medium, high, respectively, mean values for the concerned trait in the respective line. The X- and Y-axes are divided into low, medium and high sections ; this divides the graph into nine sections.

character occupies a definite position on the glyph. The range of variation in a character is represented by the length of the corresponding ray. For convenience, the mean values for each trait are classified into three groups, *viz.*, low (index score, 1), medium (score, 2), and high (score, 3). The length of each ray, as a result, is either short (low mean value), medium (medium mean value) or long (high mean value). The worth of an individual line is assessed from the sum of index values for all the characters represented in the graph. The maximum and the minimum scores that an individual line can get will be $3n$ and n, respectively, where n is the total number of characters studied.

The X- and Y-axes are also demarcated into low, medium and high mean values. This divides the entire graph into nine quadrangles, each quadrangle representing one variability group. The variation is studied within a group as well as among the groups. This technique has been used for the analysis of variability in various crops.

AIDS TO SELECTION

Selection is perhaps the most important activity in all plant breeding programmes. The various types of selection schemes, *viz.*, mass selection, progeny selection, and cyclic selection, are used depending on the mode of pollination of the crop species, the predominant gene action, and the breeding objective. Selection is practised both in homozygous as well as segregating populations. The efficiency of selection largely depends on the extent of genetic variability present in the population, and the heritability of the concerned character. *Selection is generally more effective for characters with high heritability than those having low heritability.*

Higher yields are the breeding objective in all the crops, but generally yield has low heritability. Yield is regarded as a *complex character* or *super character*, which is influenced by many component or contributing traits both in positive and negative directions. Generally, direct selection for yield is not sufficiently effective due to its low heritability, and it is desirable to select indirectly for improved yield. Some biometrical techniques provide information about the relative contributions of the various component traits to yield. Therefore, these techniques aid in the isolation from populations of superior yielding genotypes by providing information for indirect selection for yield. These techniques include, (1) correlation coefficient, (2) path coefficient, and (3) discriminant function analyses.

Correlation Coefficient Analysis. The statistics which measures the relationship between two or more variables is known as *correlation coefficient*. Correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters on which selection can be based for improvement in yield. Mass selection has been used to improve grain yield through indirect selection for highly heritable traits which are associated with yield. Correlation coefficients are of three types : (1) simple or total, (2) partial, and (3) multiple correlations.

1. **Simple Correlation.** It is the association between any two variables. Simple correlations can be estimated from both unreplicated as well replicated data using the following formulae.

In case of *unreplicated data*,

$$r = C_{ov}\, x.\, y/(V_x.\, V_y)^{1/2}$$

where,

$$C_{o_v}\, x.y = [\Sigma x.y - \{(\Sigma x)(\Sigma y)/n\}]/(N-1)$$

$$V_x = s_x^2 = [\Sigma x^2 - \{(\Sigma x)^2/N\}]/(N-1)$$

$$V_y = s_y^2 = [\Sigma y^2 - \{(\Sigma y)^2/N\}]/(N-1)$$

$\Sigma x.y$ = sum of the products of all the observations on the x and y variables,

Σx = sum of all the observations on the variable x,

Σy=sum of all the observations on the variable y,

Σx^2, Σy^2=the sums of squares of all the observations on x and y variables, respectively, and

N=the number of observations on x and y variables.

Similarly, in the case of *replicated data*,

$$r = MSP_t/(MS_{t_x} MS_{t_y})^{1/2}$$

where, MSP_t=The mean sum of products for treatments (lines, varieties, strains) for the variables x and y,

MS_{t_x}=Mean squares for treatments for the variable x, and

MS_{t_y}=Mean squares for treatments for the variable y.

The estimates of MSP_t, MS_{t_x} and MS_{t_y} are obtained from analyses of covariance (for x and y variables) and variance (separately for variables x and y) tables based on the replicated data.

The simple correlation is of three types : (1) phenotypic, (2) genotypic, and (3) environmental. *Phenotypic correlation* is the observable correlation between two variables ; it includes both genotypic and environmental effects. *Genotypic correlation*, on the other hand, is the inherent association between two variables ; it may be either due to pleiotropic action of genes, linkage or more likely, both. Similarly, *environmental correlation* is entirely due to environmental effects.

These three types of correlations can be estimated from replicated data only. Following the analyses of variances and covariance of such data, phenotypic, genotypic and environmental variances and covariances are estimated in the same manner as described for variances in Chapter 4. Now, phenotypic, genotypic and environmental correlation coefficients are estimated according to the following formulae.

$$r_p = P\,Cov\,x\,.\,y/(PV_x.PV_y)^{1/2}$$
$$r_g = G\,Cov\,x\,.\,y/(GV_x\,.\,GV_y)^{1/2}, \text{ and}$$
$$r_e = E\,Cov\,x\,.\,y/(EV_x\,.\,EV_y)^{1/2}$$

where, r_p, r_g and r_e are phenotypic, genotypic and environmental, respectively, correlation coefficients ; $P\,Cov\,x\,.\,y$, $G\,Cov\,x\,.\,y$ and $E\,Cov\,x\,.\,y$ are phenotypic, genotypic and environmental, respectively, covariances between variables x and y ; PV_x, GV_x and EV_x are phenotypic, genotypic and environmental, respectively, variances for the variable x ; and PV_y, GV_y and EV_y are phenotypic, genotypic and environmental, respectively, variances for the variable y.

2. Partial Correlation. When the correlation between two variables, say, X_1 and X_2, is estimated by taking into account the effect of a third variable, *e.g.*, X_3, it is called *partial* or *net*

correlation, and is denoted as $r_{12 \cdot 3}$. It provides a better insight into the true relationship between the two variables X_1 and X_2 than is available from the estimate of simple correlation coefficient between them. It is calculated from the estimate of simple correlation coefficients according to the following formula.

$$r_{12 \cdot 3} = (r_{12} - r_{13} \cdot r_{23}) / \{(1 - r_{13}^2)(1 - r_{23}^2)\}^{1/2}$$

where, r_{12}, r_{13} and r_{23} are the estimates of simple correlation coefficients between the variables X_1 and X_2, X_1 and X_3, and X_2 and X_3, respectively.

3. Multiple Correlation. The estimate joint influence of two or more variables on a dependent variable is called *multiple correlation*. Such an estimate helps in understanding the dependence of one variable, X_1, on a set of independent variables, say, X_2, X_3 etc. It is denoted as $R_{1 \cdot 23}^2$, where, R^2 is the coefficient of multiple correlation, 1 is the dependent variable X_1, and 2 and 3 are the independent variables X_2 and X_3. It measures the joint influence of the independent variables X_2 and X_3 on the dependent variable X_1. The multiple correlation coefficient is estimated from the estimates of simple correlation coefficient as follows.

$$R^2_{1 \cdot 23} = (r_{12}^2 + r_{13}^2 - 2r_{13} \cdot r_{12} \cdot r_{23}) / (1 - r_{23}^2)$$

The equare root $R^2_{1 \cdot 23}$ is the estimate of multiple correlation coefficient. The coefficient of determination can be estimated as the square of the coefficient of multiple correlation. Thus,

$$\text{Coefficient of determination} = R^2_{1 \cdot 23}$$

Path Analysis. The concept of path coefficient analysis was originally developed by Wright in 1921, but the technique was first used for plant selection by Dewey and Lu in 1959. Path analysis is simply standardized partial regression coefficient which splits the correlation coefficients into the measures of direct and indirect effects of a set of independent variables on the dependent variable. For example in black gram, grain yield (X_5) is affected by the number of primary branches (X_1), secondary branches (X_2) pods per plant (X_3) and seed per pod (X_4). The path analysis unravels whether the association of these characters with yield is due to their direct effect on yield, or is a consequence of their indirect effect *via* some other trait(s). If the correlation between yield and a character is due to the direct effect of character, it reflects a true relationship between them and selection can be practised for such a character in order to improve yield. But if the correlation is mainly due to indirect effect of the character through another component trait, the breeder has to select for the latter trait through which the indirect effect is exerted.

Path analysis is carried out using the estimates of correlation coefficients. To begin with, all possible correlations among the dependent and independent variables are worked out. Now the path analysis is undertaken in three steps, *viz.*, calculation of (1) direct effects, (2) indirect effects and (3) the residual effect.

1. Direct Effect. Every component character will have a direct effect on yield. In addition, it will also exert indirect effect *via* other component characters. In order to estimate the direct effects of different component traits, first a path diagram is constructed as follows (Fig. 5.2), using the estimates of correlation coefficients. With the help of the path diagram, simultaneous equations are developed as follows.

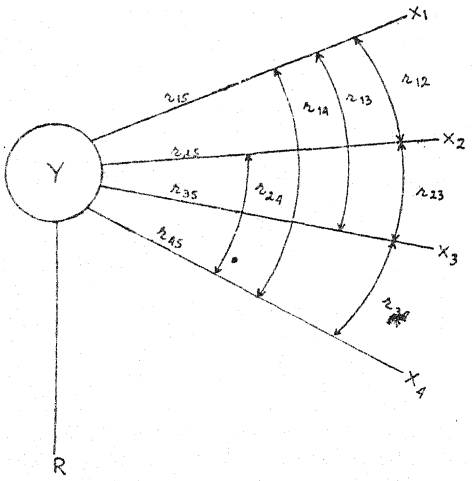


Fig. 5.2. A path diagram constructed using the correlation coefficients among yield (Y) and four of its component traits in black gram. R denotes the residual effect.

$$r_{15}=P_{15}+r_{12} \cdot P_{25}+r_{13} \cdot P_{35}+r_{14} \cdot P_{45}$$
$$r_{25}=r_{12} \cdot P_{15}+P_{25}+r_{23} \cdot P_{35}+r_{24} \cdot P_{45}$$
$$r_{35}=r_{13} \cdot P_{15}+r_{25} \cdot P_{25}+P_{35}+r_{34} \cdot P_{45}, \text{ and}$$
$$r_{45}=r_{14} \cdot P_{15}+r_{24} \cdot P_{25}+r_{34} \cdot P_{35}+P_{45}$$

where, r_{12}, r_{13}, r_{14}, etc. are the estimates of correlation coefficients between variables X_1 and X_2, X_1 and X_3, X_1 and X_4, etc., respectively, and P_{15}, P_{25}, P_{35} and P_{45} are the estimates of the direct effects of variables X_1. X_2, X_3 and X_4, respectively, on the dependent variable X_5 (in this case yield). After putting the values of the correlation coefficients in these equations, the values of P_{15}, P_{25} etc. are estimated by the process of elimination.

2. Indirect Effects. These effects are computed by putting the values of correlation coefficients and those of direct effects as follows.

Indirect effect of primary branches (X_1) *via*,

secondary branches	(X_2)	$=r_{12} \cdot P_{25}$
pods per plant	(X_3)	$=r_{13} \cdot P_{35}$
seeds per pod	(X_4)	$=r_{14} \cdot P_{45}$

Similarly, the indirect effects of secondary branches (X_2) will be as follows.

via primary branches (X_1) $= r_{12} \cdot P_{15}$
via pods per plant (X_3) $= r_{23} \; P_{35}$
via seeds per pod (X_4) $= r_{24} \cdot P_{45}$

The indirect effects of the other component traits, *e.g.*, pods per plant (X_3) and seeds per pod (X_4), may be computed in a similar fashion.

3. **Residual Effect.** Using the values of direct effects and correlation coefficients, the residual effects are estimated according to the following formula, the equation is based on the earlier example of black gram.

$$1 = P^2R_5 + P_{15} \cdot r_{15} + P_{25} \cdot r_{25} + P_{35} \cdot r_{35} + P_{45} \cdot r_{45}$$

where, P^2R_5 is the square of residual effect.

Information obtained from path analysis has been extensively used in different crops for indirect selection for yield. For example in oats, indirect selection through seed width resulted in a 9% increase in grain yield. Selection for a component trait with a view to improve yield is called *indirect selection*, while selection for yield *per se* is termed as *direct selection*. In maize, indirect mass selection for number of ears per plant resulted in an increased yield. A greater yield response is obtained when the character for which indirect selection is practised has a high heritability and a high correlation with yield. The minimum combinations of heritability and correlation coefficient values necessary for indirect selection to be more efficient than direct selection for yield itself have been tabulated (see, Moll and Stuber, 1974).

Discriminant Function. The use of discriminant function for plant selection was first proposed by Smith in 1936. He suggested that a better way of exploiting genetic correlation with several traits having high heritability is to construct an index, called *selection index*, which combines information on all the characters associated with the dependent variable, yield. Selection index involves a selection criterion based on a combination of measurements on various characters. The best known selection indices involve discriminant functions based on the relative economic importance of the various characters. This technique provides information on yield components and thus aids in indirect selection for the improvement of yield. Since the desirable genotypes are discriminated from the undesirable ones, based on the combinations of various characters, this technique is known as *discriminant function analysis*. Later on in 1943, Hazel developed a simultaneous selection model based on the approach of path analysis ; subsequently several modifications were made in the technique by different workers for specific breeding requirements.

It has been pointed out that there exists some uncertainty in the usefulness of this technique due to the errors in estimation of the statistics upon which the index weights are based. In order to solve some of the problems associated with the estimations, in 1957

Hanson and Johnson proposed a modification of the scheme of Smith in which the weights for various traits are based on the average statistics for several populations. An experimental comparison of these two types of indices (as per Smith and according to Hanson and Johnson) for soybean yields shows that only the specific index as proposed by Smith was more effective than direct selection for yield. However, it is doubtful that indices are sufficiently more effective than the selection for yield alone in order to justify the extra effort required in constructing them.

Selection indices are generally classified into three categories : (1) classical, (2) restricted, and (3) general. The estimation of all these indices is based on the estimates of phenotypic and genotypic variances and covariances of the characters involved in the index. Worked examples of the three models are available in Singh and Chaudhary (1985).

AID TO THE CHOICE OF PARENTS AND BREEDING PROCEDURES

Hybridization is the most potent technique for breaking yield barriers and evolving varieties having a built in high yield potential. The selection of suitable parents for hybridization is one of the most important steps in a hybridization programme. Selection of the parents on the basis of phenotypic performance alone is not a sound procedure since phenotypically superior lines may yield poor recombinants in the segregating generations. It is, therefore, essential that parents should be chosen on the basis of their genetic value. There are several techniques for the evalution of varieties or strains in terms of their genetic makeup. Of these, diallel, partial diallel and line × tester techniques are in common use. A brief description of these and some other techniques is given below.

Diallel Cross Analysis. *Diallel cross* refers to all possible crosses among *n* lines, and the analysis of such a set of crosses is known as *diallel analysis.* Diallel analysis is based on the following seven assumptions (Hayman, 1954).

1. Normal diploid segregation.
2. Lack of maternal effects.
3. Absence of multiple alleles.
4. Homozygosity of parents.
5. Absence of linkage among genes affecting the character.
6. Lack of epistasis.
7. Random mating.

There are two approaches for diallel analysis : (1) Hayman's approach and (2) Griffing's approach.

1. Hayman's Graphical Approach. This approach is based on the estimation of components of variation. It was initially developed by Jinks and Hayman in 1953, and later elaborated

independently by Jinks and Hayman. The following six components of variation are estimated.

D = additive genetic variance.

H_1 = dominance variance.

H_2 = $H_1[1-(u-v)^2]$, where, u and v are the proportions of positive and negative genes, respectively, in the parents.

E = expected environmental component of variance.

F = mean of Fr over the arrays, where, Fr is the covariance of additive and dominance effects in a single array.

h^2 = dominance effect, as algebric sum over all the loci in heterozygous phase in all the crosses.

From these estimates, the following genetic ratios are determined.

1. Average degree of dominance $(=H_1/D)^{1/2}$. If the value of this ratio is zero, there is no dominance ; if it is greater than zero but less 1, there is partial dominance ; if it is equal to 1, there is complete dominance ; and if it is greater than 1, it denotes overdominance.
2. The ratios of dominant and recessive genes in the parents is estimated as follows,

$$[(4\ DH_1)^{1/2} + F] \,/\, [(4DH_1)^{1/2} - F]$$

If this ratio is 1, the dominant and recessive genes in the parents are in equal proportion ; if it is less than 1, it indicates an excess of recessive genes ; but if is it greater than one, an excess of dominant genes is indicated.

3. The number of gene groups which control the character and exhibit dominance is given by,

$$h^2/H_2$$

4. The proportion of genes with positive and negative effects in the parents is estimated as,

$$H_2/4H_1$$

If the positive and negative alleles are symmetrically distributed, this ratio equals 0.25.

The above components are estimated from the data on F_1 generation of a diallel cross. In 1956, Jinks gave the procedure for estimating these components from F_2 data as well. The coefficients of H_1 and H_2 are $\frac{1}{4}$ in F_2, while they are 1 in F_1. The h and F have a coefficient of $\frac{1}{2}$ because of the one generation of inbreeding to obtain F_2. Accordingly, the genetic ratios in F_2 are worked out as follows.

1. Degree of dominance $= [\frac{1}{4}(H_1 \,/\, D)]^{1/2}$
2. Proportion of dominant and recessive genes in the parents

$$=[\tfrac{1}{4}\{(4DH_1)^{1/2} + \tfrac{1}{2}F\}/\{\tfrac{1}{4}\ (4DH_1)^{1/2} - \tfrac{1}{2}F\}]$$

The proportion of genes with positive and negative effects in the parents $(=H_2/4H_1)$, and the number of groups of genes which

control the character and exhibit dominance ($=h^2/H_2$) are the same in F_2 as in F_1.

V_r-W_r Graph. V_r is the variance of rth array and W_r is the covariance between the parents and the offspring in the rth array. The relationship of $V_r - W_r$ provides some useful information ; it is generally depicted graphically (Fig. 5.3). Some of the inferences that can be drawn from such a graph are as follows.

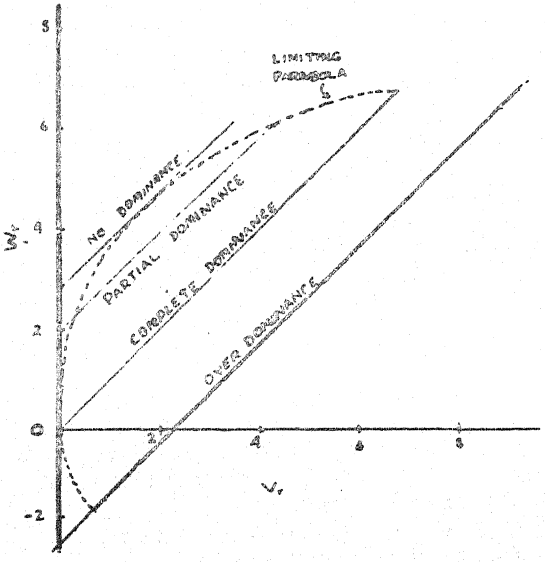


Fig. 5.3. Interpretations of the $V_r - W_r$ graph depending on the path traversed by the regression line. The limiting parabola (dotted line) joints the points of the arrays whose common parents contain all the dominant or all the recessive alleles.

1. When the regression line passes through the origin, it indicates complete dominance ($D=H_1$).
2. When it passes above the origin, cutting the W_r axis, it shows that there is partial dominance ($D>H_1$).
3. When it passes above the origin, cutting the W_r axis and touching the limiting parabola, it suggests the absence of dominance.
4. But when it passes below the origin, cutting the V_r axis, it denotes the presence of overdominance.
5. The position of parental points on the regression line indicates the dominance order of the parents. The parents with more dominant genes are located nearest to the origin, while those with more recessive genes fall farther from the origin. The parents with equal frequencies of dominant and recessive genes occupy an intermediate position.

2. Griffing's Numerical Approach. This approach is based on the estimation of *gca* (general combining ability) and *sca* (specific combining ability) variances and effects. Griffing (1956) has given four different methods for diallel analysis depending on the material included in the experimentation as shown below (Table 5.1).

Table 5.1. The four different types of diallel sets, involving *n* parents, for which Griffing (1956) has outlined the procedure for combining ability analysis.

Material included in the experiment	*Number of entries in the experiment (F_1's + parents)*
Parents, F_1's and reciprocals	n^2
Parents, and F_1's (without reciprpcals)	$n(n+1)/2$
F_1's and reciprocals (without parents)	$n(n-1)$
F_1's (without parents and reciprocals)	$n(n-1)/2$

In this approach, gene action is deduced through the estimates of *gca* (general combining ability) and *sca* (specific combining ability) variances and effects. The *gca* component is primarily a function of the additive genetic variance. But if epistasis is present, *gca* will include the additive × additive interaction component as well. On the other hand, *sca* variance is mainly a function of dominance variance, but it would include all the three types of epistatic interaction components, if epistasis is present.

Diallel analysis has been extensively used in both self- and cross-pollinated species to understand the nature of gene action involved in the expression of quantitative traits. The merits and demerits of diallel analyses are summarised below.

Merits. It provides a sensitive approach to large scale studies of quantitative characters. It yields reliable information on the components of variance, and on *gca* and *sca* variances and effects. Thus it helps in the selection of suitable parents for hybridization as well as in the choice of appropriate breeding procedures.

Demerits. This technique can test only a limited number of parents at a time. This is because for every increment of 1 in the number of parents (n) the number of crosses increases by $2n-2$ in the case of a diallel set including the reciprocals. Many scientists feel that in self-pollinated crops only the combining ability analysis should be carried out. In any case, all the assumptions of the diallel are seldom fulfilled; the assumptions of absence of linkage and epistasis are generally not satisfied. Procedures for handling the diallel analysis in the presence of epistasis have been proposed; one simple approach is to eliminate the parent and its array that is interacting.

Partial Diallel Analysis. The concept of partial diallel was developed by Kempthorne in 1957 and was further elaborated by

Kempthorne and Curnow in 1961, and by several others. It is sometimes termed as fractional diallel. It may be defined as a number of sampled crosses per parent or per array in all possible combinations of a given set of parents. The total number of sampled crosses is $ns/2$, where, n is the number of parents and s is the number of sampled crosses per parent or array. The s should be either greater than or equal to $n/2$. Clearly, both n and s can neither be odd nor even. If n is odd, s should be even and *vice-versa*.

1. Sampling Procedure. For sampling, first a constant K is worked out as follows.

$$K=(n+1-s)/2$$

If $n=10$, and $s=5$, K will be $=(10+1-5)/2=3$. This means that in each array, five crosses are to be made ($s=5$), and sampling is to begin after 3 arrays, *i.e.*, from the 4th array, as depicted below (Table 5.2). In this partial diallel, only 25 crosses [$=ns/2=(10\times5)/2=25$) will be included.

Table 5.2. The procedure for sampling for a partial diallel set when $n=10$ and $s=5$; here, $K=(10+1-5)/2=3$. The crosses included in the partial diallel are marked by ×.

Parents	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
P_1				×	×	×	×	×		
P_2					×	×	×	×	×	
P_3						×	×	×	×	×
P_4							×	×	×	×
P_5								×	×	×
P_6									×	×
P_7										×
P_8										
P_9										
P_{10}										

With the partial diallel approach, a large number of parents can be evaluated at the same time. Let us suppose, 20 parents are involved ; in a partial diallel, only $(20\times11)/2=110$ (here, $n=20$, $s=11$) have to be made, while in a complete diallel set, including the reciprocals, 20 $(20-1)/2=190$ crosses must be made ; this is almost twice as much work. The partial diallel analysis provides information about *gca* and *sca* variances and *gca* effects, and on the components of genetic variance (σ^2A and σ^2D), but it fails to estimate *sca* effects.

Triallel Analysis. A triallel cross is a three-way cross or a complex cross involving three parents, *viz.*, (A×B) C. The all possible three-way crosses among n lines will be,

$${}^nc_3=3\,[n\,!\,/\,(n-3)\,!\,(3\,!\,)\,]$$

$$=3\left[\frac{n\,(n-1)\,(n-2)\,(n-3)\,!}{(n-3)\,!\,(3\times2\times1)}\right]$$
$$=[n\,(n-1)\,(n-2)\,]/2$$

The concept of triallel analysis was developed by Rawlings and Cockerham in 1962. This analysis provides information on epistatic components of the variance (σ^2AA, σ^2AD, σ^2DD) as well. It also yields information on the order in which the parents should be crossed for obtaining superior segregants. It has been shown that the variance components in a series of three way crosses will exhibit the following genetic components.

$$\sigma^2 \text{ three-way crosses}=3/4\ \sigma^2A+1/2\ \sigma^2D+9/16\ \sigma^2AA+ 3/8\ \sigma^2AD+1/4\ \sigma^2DD+.....$$

Quadriallel Analysis. The concept of quadriallel analysis was developed by Rawlings and Cockerham in 1962. It is an analysis of double-cross hybrids, which are the first generation progeny of a cross between two unrelated F_1 hybrids, *e.g.*, (A×B) (C×D), where, A, B, C and D are the four parents, and A×B and C×D are the two unrelated F_1 hybrids involving these parents. Thus quadriallel analysis requires two cropping seasons for generating the experimental material (as is the case for triallel as well), and is more demanding than the triallel analysis in terms of the number of crosses that have to be effected.

In order to generate the material, the F_1's among the parental lines are produced in a diallel fashion : the F_1's are then mated according to the diallel scheme, with the restriction that no parent should appear more than once in the same double-cross combination, to produce the double-cross hybrids. For example, if the number of parents is 6, there will be 15 [=(6×5)/2] different single-cross hybrids among them (excluding the reciprocals). These single-crosses when mated in a diallel fashion, subject to the restriction mentioned above, will generate,

$$3\ ({}^nc_4)=3\ [n\ !/(4\ !)\ (n-4)\ !\ ] \text{ double crosses.}$$

where, n is the number of the parents involved. If $n=6$, the number of all possible double-cross combinations will be,

$$=3\ [n\ !\ /\ (4\ !)\ (n-4\ !\ )=3\ [6\ !\ /\ (4\ !)\ (6-4)\ !\ ]=45$$

This technique offers the same additional information over the other schemes as does the triallel analysis. It provides more estimates of the components genetic variance of epistatic nature and also yields information on the order effects of the combinations in double-cross hybrids. Variance components in a series of double-crosses show the following genetic variance.

$$\sigma^2 \text{ double-crosses}=1/2\ \sigma^2A+1/4\ \sigma^2D+1/4\ \sigma^2AA+ 1/8\ \sigma^2AD+1/16\ \sigma^2DD+.....$$

Line × Tester Analysis. *Line × tester cross* is a modified form of the top-cross scheme proposed by Davis in 1962. The line × tester

technique was developed by Kempthorne in 1957. It is a good approach for screening the germplasm on the basis of *gca* and *sca* variances, and effects. The total number of crosses to be made is equal to the product of the number of lines and the number of testers included in the study. Thus,

$$\text{total number of crosses} = n \cdot t$$

where, n is the number of lines and t is the number of testers. If there are 30 lines and 3 testers, the total number of crosses will be 90 ($=30 \times 3$).

Several types of testers have been suggested by different authors. Some feel that (1) heterozygous testers are superior to homozygous ones. (2) The best inbred line has a masking effect due to its desirable dominant alleles ; therefore, it should not be used as a tester. Thus in order to obtain the real expression of a line, low performing testers should be used. (4) An inferior synthetic developed by crossing together poor lines may be used as a tester. Thus a synthetic susceptible to lodging should be used to test the capacity of lines to withstand lodging. In general, a tester should be poor in the traits for which the lines are to be analysed. Further, the testers should be highly adaptable to environmental fluctuations.

The line × tester technique has been extensively used in almost all the major field crops to estimate *gca* and *sca* variances and effects, and to understand the nature of gene action involved in the expression of various quantitative traits. This technique measures the *gca* and *sca* variances and effects and the genetic components of variance ($\sigma^2 A$ and $\sigma^2 D$). It however, fails to detect and estimate the epistatic variance.

Generations Mean Analysis. This analysis is based on six populations, P_1, P_2, F_1, F_2, B_1 and B_2. Hayman (1958) and Jinks and Jones (1958) developed the six parameter model for the estimation of various components of genetic variance. Hayman (1958) has given the procedure for estimating the various gene effects as follows.

m = mean effect $= \bar{F}_2$.

d = additive effect $= \bar{B}_1 - \bar{B}_1$.

h = dominance effect $= \bar{F}_1 - 4\bar{F}_2 - \frac{1}{2}\bar{P}_1 - \frac{1}{2}\bar{P}_2 + 2\bar{B}_1 + 2\bar{B}_2$

i = additive × additive gene interaction

$= 2\bar{B}_1 + 2\bar{B}_2 - 4\bar{F}_2$

j = additive × dominance gene interaction

$= \bar{B}_1 - \frac{1}{2}\bar{P}_1 - \bar{B}_2 + \frac{1}{2}\bar{P}_2$

l = dominance × dominance gene interaction

$= \bar{P}_1 + \bar{P}_2 + 2\bar{F}_2 + 4\bar{F}_2 - 4\bar{B}_1 - 4\bar{B}_2$

where, $\bar{P}_1$, $\bar{P}_2$, $\bar{F}_1$, $\bar{F}_2$, $\bar{B}_1$ and $\bar{B}_2$ are the mean values for the character in the P_1, P_2, F_1, F_2, B_1 and B_2 populations, respectively.

The variances for these gene effect estimates are obtained by the following formulae :

$$Vm = V\bar{F}_2$$

$$Vd = V\bar{B}_1 + V\bar{B}_2$$

$$Vh = V\bar{F}_1 + 16\,V\bar{F}_2 + \tfrac{1}{4}\,V\bar{P}_1 + \tfrac{1}{4}\,V\bar{P}_2 + 4\,V\bar{B}_1 + 4\,V\bar{B}_2$$

$$Vi = 4\,V\bar{B}_1 + 4\,V\bar{B}_2 + 16\,V\bar{F}_2$$

$$Vj = V\bar{B}_1 + 1/4\,V\bar{P}_1 + V\bar{B}_2 + 1/4\,V\bar{P}_2$$

$$Vl = V\bar{P}_1 + V\bar{P}_2 + 4\,V\bar{F}_1 + 16\,V\bar{F}_2 + 16\,V\bar{B}_1 + 16\,V\bar{B}_2$$

In the absence of nonallelic interactions, Jinks and Jones (1958) used the following formulae for the estimation of *m*, *d* and *h* (commonly known as the *three parameter model*).

$$m = 1/2\,\bar{P}_1 + 1/2\,\bar{P}_2 + 4\,\bar{F}_2 - 2\,\bar{B}_1 - 2\,\bar{B}_2$$

$$d = Y_2\ 1/2\,\bar{P}_1 - 1/2\,\bar{P}_2$$

$$h = 6\,\bar{B}_1 + 6\,\bar{B}_2 - 8\,\bar{F}_2 - \bar{F}_1 - 3/2\,\bar{P}_1 - 3/2\,\bar{P}_2$$

The variances for these estimates are calculated as follows.

$$Vm = 1/4\,V\bar{P}_1 + 1/4\,V\bar{P}_2 + 16\,V\bar{F}_2 + 4\,V\bar{B}_1 + 4\,V\bar{B}_2$$

$$Vd = \tfrac{1}{4}\,V\bar{P}_1 + \tfrac{1}{4}\,V\bar{P}_2$$

$$Vh = 36\,V\bar{B}_1 + 36\,V\bar{B}_2 + 64\,V\bar{F}_2 + V\bar{F}_1 + \tfrac{9}{4}\,V\bar{P}_1 + \tfrac{9}{4}\,V\bar{P}_2$$

When F_2 is included and the backcrosses (B_1 and B_2) are absent, five parameters, *viz.*, *m*, *d*, *h*, *i* and *l* can be estimated as follows.

$$m = \bar{F}_2$$

$$d = \tfrac{1}{2}\,\bar{P}_1 - \tfrac{1}{2}\,\bar{P}_2$$

$$h = \tfrac{1}{6}\,(4\,\bar{F}_1 + 12\,\bar{F}_2 - 16\,\bar{F}_3)$$

$$i = \bar{P}_1 - \bar{F}_2 + \tfrac{1}{2}\,(\bar{P}_1 - \bar{P}_2 + h) - \tfrac{1}{4}\,l$$

$$l = \tfrac{1}{3}\,(16\,\bar{F}_3 - 24\,\bar{F}_2 + 8\,\bar{F}_1)$$

The variances for these estimates are computed as follows.

$$Vm = V\bar{F}_2$$

$$Vd = \tfrac{1}{4}\,(V\bar{P}_1 + V\bar{P}_2)$$

$$Vh = \tfrac{1}{16}\,(16\,V\bar{F}_1 + 144\,V\bar{F}_2 + 256\,V\bar{F}_3)$$

$$Vi = V\bar{P}_1 + V\bar{F}_2 + \tfrac{1}{4}\,(V\bar{P}_1 + V\bar{P}_2 + V\,h) + \tfrac{1}{16}\,V\,l$$

$$Vl = \tfrac{1}{9}\,(256\,V\bar{F}_3 + 576\,V\bar{F}_2 + 64\,V\bar{F}_1)$$

Mather estimated three components of the variance in the absence of epistasis as follows (see, Chapter 4).

Heritable fixable variance $(D) = 4\,VF_2 - 2\,(VB_1 + VB_2)$

Heritable nonfixable variance $(H) = 4\,(VB_1 + VB_2 - VF_2 - VE)$

Non-heritable nonfixable variance $(E) = (VP_1 + VP_2 + VF_1)/3$

The genetic advance, heritability and inbreeding depression can be calculated as given below.

Genetic advance $(Gs)=[VG/(VP)^{1/2}]\times k$

where, k is a constant based on selection intensity, $VG=VF_2-VE$, and $VP=VF_2$. Hence,

$$Gs=[(VF_2-VF_1)(VF_2)^{1/2}]\times k$$

And heritability, in broad sense, is estimated as follows.

Heritability (in broad sense) $=[(VF_2-VF_1)/VF_2]\times 100$

while, heritability (in narrow sense) $=(\frac{1}{2}\ D/VF_2)\times 100$ (Warner, 1952)

or $=[D/(D+H+E)]\times 100$ (Mather, 1949)

Further, inbreeding depression $=[(\bar{F}_1-\bar{F}_2)/\bar{F}_1]\times 100$

The above techniques for estimating the components of variance provide information about the predominant type of gene action for the important traits of a crop species. This helps in deciding on a suitable breeding procedure for the improvement of various quantitative traits of the species.

Biparental Cross Analysis. The concept of biparental mating was originally developed by Comstock and Robinson in 1948 and 1952. In this technique, plants are randomly selected in F_2 or a subsequent generation of a cross between two purelines having contrasting performance ; the selected plants are crossed according to a definite scheme (see below). Biparental crosses include full-sib and half-sib progenies in the mating programme. Two important genetic parameters, *i.e.*, additive (σ^2A) and dominance (σ^2D) genetic variance are estimated from biparental crosses. However, this analysis does not provide any information on the epistatic component. The biparental cross analysis is based on the following eight assumptions.

1. Random distribution of genotypes in relation to variation.
2. Random choice of plants for mating.
3. Regular diploid segregation.
4. Absence of maternal effects.
5. Lack of multiple allelism.
6. Absence of epistasis.
7. Absence of linkage.
8. Equal surrvival of all genotypes.

Three mating designs for biparental crosses, commonly known as *North Carolina Designs I, II* and *III* (NCD I, II and III, respectively) have been proposed. A brief description of these designs is given below.

1. **North Carolina Design I.** In this design, five plants are randomly selected from an F_2 or a subsequent generation of a cross. One of these plants is designated as male and crossed with each of the remaining four plants, which are referred to as females. The set of four full-sib families thus produced is

denoted as a *male group*; four such male groups (16 female groups) constitute one *set*. A *female group* consists of one full-sib family produced by crossing one female plant with one male plant. In this scheme, a female plant (or animal) is used for only one mating, while each male is mated to four different females. The number of females mated to each male may be more than four and may vary from one male to the other, but usually it is kept uniform for ease in statistical treatment. The design separates the variance of progenies into two fractions, *viz.*, (1) variance due to males ($\sigma^2 m$), which is equal to $\frac{1}{4}\sigma^2 A$, and (2) variance due to females ($\sigma^2 f$), which equals $\frac{1}{4}\sigma^2 A + \frac{1}{4}\sigma^2 D$.

2. **Notrh Carolina Design II.** According to NCD II, equal number of males (or plants so designated) and females (or plants so designated) are randomly selected from an F_2 population, and each male is crossed with each female. Thus the total number of crosses produced will be $m \times f$, where m is the number of males, f is the number of females, and $m=f$. In this design, both maternal and paternal half-sibs are produced. This design separates the variance of progenies into the following three fractions: (1) variance due to males ($=\frac{1}{4}\sigma^2 A$), (2) variance due to females ($=\frac{1}{4}\sigma^2 A$) and (3) variance due to males $\times$ females ($=\frac{1}{4}\sigma^2 D$).

3. **North Carolina Design III.** In this design (NCD III), several plants are randomly selected from an F_2 population, and are designated as males. Each male plant is backcrossed to both the parents involved in the cross to produce pairs of backcross progenies. This design separates the variance into two fractions, namely, (1) variance among males ($=\frac{1}{4}\sigma^2 A$), and (2) variance due to males $\times$ females ($=\frac{1}{2}\sigma^2 D$).

Of the three North Carolina Designs, NCD III is the most useful as it requires a relatively smaller experimental area, and does not depend on any assumption about gene frequencies. Further, the analysis of variance is not influenced by the presence of maternal effects. NCD I and NCD II require 10-12 and 2-4 times, respectively, as much area as NCD III.

The randomly selected plants from an F_2 generation may also be mated either in pairs or in a diallel fashion. But the information available from these two mating schemes is rather meagre, and they are not in common use.

4. **Triple Test Cross Analysis.** The concept of triple test cross (TTC) analysis was developed by Kearsey and Jinks in 1968. It refers to the crossing of randomly selected F_2 plants with both the parents involved in the cross, and with their F_1 hybrid. Thus it is an extension of the North Caroline Design III of biparental matings. Triple test cross provides information about the presence or absence of epistasis, in addition to

estimating σ^2A and σ^2D. In this design, a random sample of n plants from an F_2 is crossed as male parent with three testers (which are used as females) ; two of the testers (L_1 and L_2) are the parental inbred lines or purelines as in the NCD III, while the third tester (L_3) is the F_1 produced by crossing these inbred testers. If the value $(\bar{L}_1+\bar{L}_2-2\bar{L}_3)$ deviates significantly from zero, the presence of epistasis is indicated, where, $\bar{L}_1$, $\bar{L}_2$ and $\bar{L}_3$ are the mean values of the progenies derived from matings with the tester L_1, L_2 and L_3, respectively.

In 1976, Ketata *et al.* proposed a somewhat similar approach in which the testers L_1, L_2 and their hybrid (L_3) are crossed with a number of unrelated strains, instead of random individuals selected from the F_2 population derived from the cross $L_1 \times L_2$, as suggested by Kearsey and Jinks in 1968. This technique is similar to TTC, has a similar statistical treatment, and yields comparable information.

STUDY OF VARIETAL ADAPTATION

Varietal adaptability to environmental fluctuations is important for the stabilization of crop production both over regions and years. *Adaptability* is the ability of a genotype to produce a relatively narrow range of phenotypes in different environments. It is the result of *genetic homeostasis*, which refers to the buffering capacity of a genotype to environmental fluctuations. The performance of a genotype mainly depends on the environmental interaction. However, in many case a linear relationship is found between performance of genotypes and the environmental conditions. Estimation of phenotypic stability, which involves regression analysis, has proved to be a valuable technique in the assessment of this linear relationship between the responses of genotypes and the environmental changes.

The first systematic approach to the analysis of phenotypic stability was made by Finley and Wilkinson in 1963. They used two parameters, namely, (1) mean performance over all the environments, and (2) regression of the performances in different environments over the respective environmental means. In this model, a regression coefficient of (*i*) unity indicates average stability, (*ii*) greater than one means below average stability, and (*iii*) less than one means the genotype has a greater resistance to environmental changes and possesses above average stability. Absolute phenotypic stability would be expressed by a regression coefficient of zero. However, in deciding about the worth of a genotype, its mean performance must be considered alongwith its phenotypic stability. Otherwise a variety which is the lowest yielding in all the environments will necessarily show a b value of less than one. Generally, a variety having $b = 1$ and a high mean yield would be considered as the most widely adapted, while a b value of one and a low mean yield (over the environments) would indicate a poorly adapted genotype.

In 1966, Eberhart and Russel made further improvements in this analysis by partitioning the genotype × environment interaction of each variety inte two parts, *viz.*, (1) slope of the regression line, and (2) deviations from the regression line. They defined a *stable variety* as one with a regression coefficient of unity ($b=1$) and a minimum deviation from the regression line ($sd^2=0$). Using their definition, a breeder would usually desire to develop a variety with high mean yield, and meets the above requirements for stability. The skeleton analysis of variance table based in this model is given below (Table 5.3).

Table 5.3. Skeleton analysis of variance table for stability as per Eberhart and Russel (1966)

Source of Variation	*d. f.*
Genotypes	$g-1$
Environment (E) + Interaction (G × E)	$g\,(e-1)$
Environment (linear)	1
G × E (linear)	$g-1$
Pooled deviations	$g(e-2)$
Genotype 1	$e-2$
Genotype 2	$e-2$
⋮	⋮
Genotype g	$e-2$
Pooled error	$g\,e\,(r-1)$

where, g, e, and r represent the numbers of genotypes, environment and replications, respectively.

In 1968, Perkins and Jinks proposed a different model for stability analysis. In this model, regression of genotypes × environments is obtained on the environmental index. In the Eberhart and Russel model, on the other hand, the variety performance is regressed on the mean of all varieties grown under a specific environmental. In the Perkins and Jinks model, the genotype × environment (G × E) sum of squares is further divided into two parts, namely, (1) sum of squares (*ss*) due to heterogeneity among regressions, and (2) *ss* due to the remainder. The First component of *ss* is the same as *ss* due to G × E (linear), while the second one is the pooled *ss* due to deviations in the Eberhart and Russel model. In this model also, regression coefficient and the deviations from regression are used as the parameters of stability. The analysis of variance is represented as follows (Table 5.4).

Table 5.4. The skeleton analysis of variance table based on the Perkins and Jinks model.

Source of variation	*d.f.*
Genotype (G)	$g-1$
Environment (joint regression)	$e-1$
Genotype × environment	$(g-1)\,(e-1)$
Heterogeneity among regressions	$g-1$
Remainder	$(g-1)\,(e-2)$
Error	$ge\,(r-1)$

where, g, e and r are the numbers of genotypes, environments and replications, respectivety.

One of the basic statistical objections to the above models of stability is the improper choice of the sum of squares and the degrees of freedom from which to subtract the regression components. In 1971, Freeman and Perkins presented a better method of partitioning the sum of squares as given below (Table 5.5).

Table 5.5. The skelton analysis of variance table based on Freeman and Perkins, 1971.

Source of Variation	*d.f.*
Genotypes (G)	$g-1$
Environments (E)	$e-1$
Combined regression	1
Residual (1)	$e-2$
Interaction (G × E)	$(g-1)(e-1)$
Heterogeneity of regressions	$g-1$
Residual (2)	$(g-1)(e-2)$
Error (between replications)	$ge(r-1)$

where, g, e and r are the numbers of genotypes, environments and replications, respectively.

In the earlier models, the estimates of mean performance and environmental index are not independent. Freeman and Perkins proposed independent estimation of these two values. In their model, the replications are divided into two groups : (1) one group is used to measure the mean performance of varieties in the different environments, while (2) the other group is used for the estimation of environmental index. Another approach for stability analysis is the use of one or more genotypes as checks to assess the environments. Although these measures provide the desired independence of the environmental and genetic effects, they entail additional experimental costs. In this model, the two residuals sums of squares form the pooled deviations.

Comstock and Moll, in 1963, defined the environment of a single organism as opposed to that of another growing at the same time in almost the same place as *micro-environment.* The environment associated with variables having large and easily recognised effects was termed as macro-environment and may include differences over years, locations, fertilizer levels, planting dates, irrigation schedules etc.

Allard and Bradshaw, in 1964, classified the environmental variation into two types : (1) predictable and (2) unpredictable. The *predictable variations* include all the permanent features of the environment, such as, soil type, day-length, planting dates, and all other agronomic practices etc. On the other hand, the *unpredictable variations* include the fluctuating features of the environment, *e.g.*, the fluctuations of weather (rainfall and temperature) etc. Therefore, planting of experiments over years and locations is less

pertinent than that over a range of agronomic practices, such as, differences in fertilizers, irrigation, sowing dates etc., which are much more pertinent measures of a crop environment.

It is generally agreed that the more stable genotypes can somehow adjust their phenotypic responses to provide some measure of uniformity in spite of environmental fluctuations. The buffering ability of the segregating populations seems to be directly related with the homeostatic responses of the parental lines. Therefore, it is feasible to develop phenotypically stable high potential genotypes by ordering homeostatic genotypes into a hybridization programme.

SUMMARY

Biometry consists of the application of statistictical concepts and techniques to the study of biological problems. *Biometrical genetics* is that branch of genetics which deals with the study of quantitative inheritance. The various statistical procedures which are used in the study of biometrical genetics are known as *biometrical techniques*. These techniques are useful to the plant breeder in four principal ways, *viz.*, (1) in the assessment of variability, (2) in the selection of elite lines, (3) in the choice of parents and breeding procedures, and (4) in the study of varietal adaptation.

Variability can be assessed in three ways : (1) by simple statistical measures, namely, range, mean, standard deviation, coefficient of variation and standard error ; (2) through D^2 statistic, and (3) with the help of metroglyph analysis. D^2 statistic measures the forces of differentiation at intra- and intercluster levels and determines the relative contribution of each component trait to the total divergence. The clusters which are separated by the largest statistical distance show the maximum divergence. *Metroglyph analysis* is a semigraphic method of studying variability in large number of germplasm lines at the same time.

Studies on correlation, path and discriminant function analyses help in the *selection of elite types*. *Correlation* measures the mutual relationship between two or more variables. Correlation is of three types : (1) simple, (2) partial and (3) multiple. *Simple correlation* is the association between two variables, and is of three types, *viz.*, (1) phenotypic, (2) genotypic, and (3) environmental. *Partial correlation* measures the association between two variables by eliminating the effect of a third variable. *Multiple Correlation*, on the other hand, measures the joint influence of two or more indedendent variables on a single dependent variable. *Path Analysis* splits the correlation coefficients into the components of direct and indirect effects. Thus it measures the direct and indirect contribution of independent variables on the dependent one. *Discriminant function analysis* involves the development of a selection criterion based on a combination of various characters, and aids in indirect selection for yield.

Three important biometrical techniques, namely, (1) diallel, (2) partial diallel, and (3) line × lester analyses are used for the *selection of desirable parents* based on the estimates of *gca* and *sca* variances and effects. There are several other techniques, *viz.*, generations mean analysis, biparental cross, triple test cross, triallel and quadriallel analyses, which are used to estimate the components of variance from different populations.

Stability reflects the suitability of a variety for a general cultivation over a wide range of environments. *Phenotypic stability* is measured by four different models given by, (1) Finley and Wilkinson in 1963, (2) Eberhart and Russel in 1966, (3) Perkins and Jinks in 1968, and (4) Freeman and Perkins in 1971. The Eberhart and Russel model is widely used as it is relatively simple yet quite informative

QUESTIONS

1. Describe briefly the various biometrical techniques which are used for assessing the variability in a collection of germplasm.
2. Some biometrical techniques are useful in providing information for indirect selection for yield. Describe these techniques in brief.
3. List and briefly describe the various techniques used for the selection of parents for hybridization programmes. Discuss their merits and demerits.
4. Differentiate between the following : (i) dominance and epistasis, (ii) correlation and path analysis, (iii) variability and diversity, (iv) adaptation and adaptability, (v) diallel and partial diallel designs.
5. Difine biparental cross. Describe the different designs used for biparental matings ; and discuss their merits and demerits.
6. Explain the different types of correlations estimated in breeding materials and discuss their implications in plant breeding.
7. What do the following terms signify ? (i) triple test cross, (ii) triallel analysis, (iii) quadriallel analysis, (iv) selection index, (v) biparental cross, (vi) line×tester analysis. (vii) $V_r - W_r$ graph.
8. Describe briefly the various methods for estimating the different components of variance.
9. List the various biometrical techniques in common use. Discuss the practical implications of any two of them in plant breeding programmes.
10. Define stability. Describe briefly the different methods used for estimating the phenotypic stability and discuss their merits and demerits.
11. Write short notes on the following : (i) biometry, (ii) D^2 statistics, (iii) correlation coefficient, (iv) path analysis, (v) components of variance, (vi) metroglyph analysis, (vii) generations mean analysis, (viii) path analysis, (ix) stability analysis.

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CHAPTER 6

Selection in Self-Pollinated Crops

In self-pollinated crops, selection permits reproduction only in those plants that have the desirable characteristics. This is achieved by raising the next generation from seeds produced by the selected plants only ; seeds from the remaining plants are rejected. Selection is essentially based on the phenotype of plants. Consequently, the effectiveness of selection primarily depends upon the degree to which the phenotype reflects the genotype. Selection has two basic characteristics or limitations. *First*, selection is effective for heritable differences only ; its effectiveness is greatly affected by heritability of the character under selection. *Second*, selection does not create new variation ; it only utilizes the variation already present in a population. *Thus the two requirements of selection are* : *(1) variation must be present in the population, and (2) the variation must be heritable.* The purpose of selection is to isolate desirable plant types from the population. Thus selection is basic to crop improvement. Indeed, it is one of the two fundamental steps of any breeding programme ; the two basic steps are : creation of variation and selection.

HISTORY OF SELECTION

Before domestication, crop species were subjected to natural selection. The basis for natural selection was adaptation to the prevailing environment. Plant tppes more adapted to the environment produced more progeny than the others. After domestication, man has knowingly or unknowingly practised some selection. Thus crop species under domestication were exposed to both natural and artificial selection, *i.e.*, selection by man. For a long period under domestication, natural selection was perhaps more important than selection by man. But in modern plant breeding methods, natural selection is of little value, and the current breeding methods depend entirely on artificial selection.

There is evidence that selection was practised by farmers in ancient times. During late eighteenth century, selection by agricul-

turists, such as Van Mons in Belgium, Andrew Knight in England and Cooper in U.S.A., resulted in several important crop varieties. Le Couteur, a farmer of the Isle of Jersey, published his results on selection in wheat in the year 1843. He concluded that progenies from single plants were more uniform than the remaining population, and that different progenies were of different agricultural value. About the same time, a Scotsman named Patrick Shireff practised individual plant selection in wheat and oats, and developed some valuable varieties.

Some years later, beginning in 1857, Hallet in England practised single plant selection in wheat, oats and barley. He believed that acquired characters were inherited. From the best plants, he selected the best spike from which he selected the best grain. Although it is now definitely known that acquired characters are not inherited, Hallet developed several commercial varieties, *e.g.*, Chevalier barley. About this time, Vilmorin proposed individual plant selection based on progeny testing. This method successfully improved the sugar content in sugarbeets (*Beta vulgaris*) in 12 years, but 50 years of selection was ineffective in improving four wheat varieties. This clearly demostrated the difference between effectiveness of selection in self- and cross-pollinated species. But the genetic basis for this difference was understood only later.

The Svalof experiment station of Swedish Seed Association, established in 1866, refined the single plant selection or *pureline selection* to its present form. Subsequently, the genetic basis of purelines was explained by Johannsen in 1903.

THE PROGENY TEST

Evaluation of the worth of plants on the basis of the performance of their progenies is known as progeny test. The progeny test was developed by Louis de Vilmorin. Therefore, it is also known as the *Vilmorin Isolation Principle* or, in short, *Vilmorin Principle.* Vilmorin observed that sugarbeet plants with high sugar content could be grouped into three classes based on the sugar content of their progenies. The *first* group of plants produced progenies high in sugar content, the progenies from the *second* group had some plants with high and some with low sugar content, while the *third* group produced progenies low in sugar content. Thus plants similar in phenotype (high sugar content) produced considerably different progenies. From these observations, Vilmorin concluded that *the real value of a plant can be known only by studying the progeny produced by it.*

Today, progeny test is the basic step in every breeding method. The progeny test serves two valuable functions. Its *first* function is to determine the breeding behaviour of a plant, *i.e.*, whether it is homozygous or heterozygous. For example, consider the progeny

from barley plants with the genotypes *VV* and *Vv*, both having two-rowed spikes. The *second*, and perhaps the more important, function of progeny test is to find out whether the character for which the plant was selected is heritable, *i.e.*, is due to genotype. Selections have to be based on phenotype. The relative contributions of genotype and environment to the phenotype of the selected plants can be determined through progeny test. If the phenotypic differences are due to differences in genotype, they will be present in the progeny as well ; otherwise they will be absent in the progeny. This will become more clear when we examine the pureline theory of Johannsen.

THE PURELINE THEORY

A pureline is progeny of a single homozygous plant of a self-pollinated species. Therefore, all the plants in a pureline have the same genotype. The phenotypic differences within a pureline are due to the environment and have no genetic basis. Therefore, variation within a pureline is not heritable.

The concept of purelines was proposed by Johannsen in 1903 on the basis of his studies with beans (*Phaseolus vulgaris*). Beans are strictly self-pollinated species. Johannsen obtained commercial seed of the Princes variety of beans. The commercial seed lot showed variation for seed size. He selected seeds of different sizes and grew them separately. The progenies thus obtained differed in seed size : progenies from larger seeds generally produced larger seeds than those obtained from smaller seeds. This clearly slowed that the variation in seed size in the commercial seed lot of Princess had a genetic basis. As a result, selection for seed size was effective. Johannsen further studied 19 lines ; each line was progeny of a single seed from the original seed lot. He discovered that each line showed a characteristic mean seed weight, ranging from 640 mg in Line No. 1 to 350 mg in Line No. 19 (Fig. 6.1). The seed size within a line showed some variation, which was much smaller than that in the original commercial seed lot. Johannsen postulated that the original seed lot was a mixture of *purelines*. Thus each of the 19 lines represented a pureline, and the variation in seed size within each of the purelines had no genetic basis and was entirely due to environment.

Confirmatory evidence was obtained in three ways. In the *first* case, he classified the seeds from each pureline into 100 mg classes, and grew them separately. The mean seed weights of progenies from different seed weight classes of a single pureline were comparable with each other, and with that of the parent pureline. For example, Line No. 13 had seed size classes of 200, 300, 400 and 500 mg. The mean seed weights of the progenies derived from these seed weight classes were 475, 450, 451 and 458 mg, respectively (Fig. 6.1).

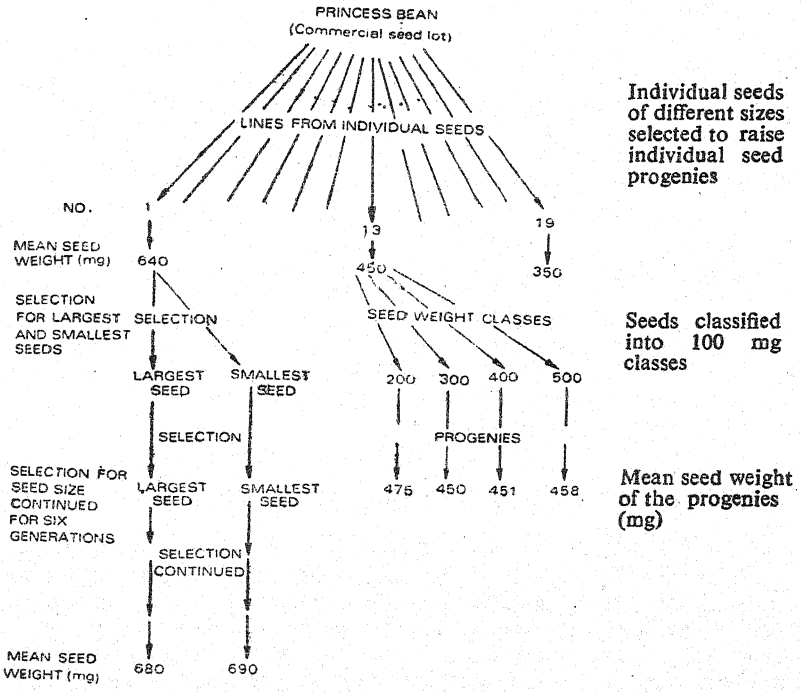


Fig. 6.1. Selection for seed weight in a commercial seed lot of the Princess variety of bean by Johannsen.

The *second* line of evidence came from selection within a pureline. From each pureline, largest and smallest seeds were selected to raise the next generation. In the subsequent generations, large seeds were selected in the progenies obtained from large seeds, while in those from small seeds selection was done for small seeds. Six generations of selection was ineffective in increasing or decreasing the seed size. For example, after six generations of selection, the mean seed weight in Line No. 1 was 690 and 680 mg in the progenies selected for small and large seeds, respectively (Fig. 6.1). Thus selection within a pureline was ineffective.

The *third* approach was to estimate parent-offspring correlation. The value of parent-offspring correlation within Line No. 13

was -0.018 ± 0.038, that is, zero, while it was 0.336 ± 0.008 in the original seed lot of the Princess which is highly significant. The parent-offspring correlation will be zero when the variation is non-heritable, while it will be significantly greater than zero when the variation has a genetic basis, *i.e.*, is heritable.

These observations reveal that the variation for seed size in the original seed lot of Princess had a genetic basis and was heritable. But the variation within the purelines obtained from the single seeds selected from this seed lot was purely due to the environment and, therefore, nonheritable. The two main conclusions from the Johannsens' experiment are,

1. A self-fertilized population consists of a mixture of several homozygous genotypes. Variation in such a population has a genetic component, and therefore selection is effective.
2. Each individual plant progeny selected from a self-fertilized population consists of homozygous plants of identical genotype. Such a progeny is known as pureline. The variation within a pureline is purely environmental and, as a result, selection within a pureline is ineffective.

EFFECTS OF SELF-POLLINATION ON GENOTYPE

Self-pollination increases homozygosity with a corresponding decrease in heterozygosity. Inbreeding also increases homozygosity and reduces heterozygosity. *Inbreeding* is mating between individuals related by descent, that is, having a common parent or parents. Some examples of inbreeding are : sib mating (brother-sister mating), half-sib mating (brother-stepsister mating) etc. Self-pollination is the most intense form of inbreeding, since in this case the same individual functions as the male as well as the female parent.

The effect of self-fertilization on homozygosity and heterozygosity may be illustrated by an example. Suppose an individual heterozygous for a single gene (*Aa*) is self-pollinated in successive generations. Every generation of self-pollination will reduce the frequency of the heterozygote *Aa* to 50 per cent of that in the previous generation. There is a corresponding increase in the frequency of the homozygotes *AA* and *aa*. As a result, after 10 generations of selfing, virtually all the plants in the population would be homozygous, *i.e.*, *AA* and *aa*. On the other hand, the frequency of the heterozygote *Aa* would be only 0.095 per cent, which is negligible (Table 6.1). It is assumed here that the three genotypes *AA*, *Aa* and *aa* have equal survival. If there is unequal survival, it may increase or decrease the rate at which homozygosity is achieved. If *Aa* is favoured, the rate of increase in homozygosity would be lower than expected. But if *Aa* is selected against, homozygosity would increase at a faster rate than expected.

Table. 6.1. Effect of self-fertilization on the frequency of homozygotes with respect to a single locus *Aa*. This also gives the *degree of homozygosity* with respect to any number of genes.

Number of generations of self-fertilization	*Frequency (%)*			*Frequency (%)*	
	AA	*Aa*	*aa*	*Homozygotes (or homozygosity)*	*Heterozygotes (or heterozygosity)*
0	0	100	0	0	100
1	25	50	25	50	50
2	(25+12.5)	25	(25+12.5)	75	25
3	(37.5+6.25)	12.5	(37.5+6.25)	87.5	12.50
4	(43.75+3.125)	6.25	(43.75+3.125)	93.73	6.25
5	(46.875+1.562)	3.125	(46.875+1.562)	96.874	3.125
6	(48.437+0.781)	1.562	(48.437+0.781)	98.436	1.562
7	(49.218+0.390)	0.781	(49.218+0 390)	99.216	0.781
8	(49.608+0.195)	0.390	(49.608+0.195)	99.606	0.390
9	(49.803+0.097)	0.195	(49.803+0.097)	99.800	0.195
10	(49.900+0.048) =49.948	0.097	(49.900+0.048) =49·948	99.896	0.097
n	$(2^{n-1}/2^{n+1})$	$(2/2^{n+1})$	$(2^{n-1}/2^{n+1})$	$\frac{2(2^{n-1})}{2^{n+1}}$ or $(2^{n-1}/2^n)$	$\frac{2}{2^{n+1}}$ $(1/2^n)$

When a number of genes are segregating together, each gene would become homozygous at the same rate as *Aa*. Thus the number of genes segregating does not affect the percentage of homozygosity. The term *homozygosity* denotes the frequency of genes in homozygous condition in the population. Similarly, linkage between genes does not affect the percentage of homozygosity in the population.

Another way of visualising the effect of self-pollination is to consider the frequency of plants which are homozygous for all the genes. In case of a single gene, the frequency of completely homozygous plants in a generation is the same as the proportion of homozygosity. But when two or more genes are segregating, the proportion of homozygosity increases at a much faster rate than that of completely homozygous plants. The proportion of completely homozygous plants is given by the following formula.

Proportion of completely homozygous plants $=[(2^m-1)/2^m]^n$ where, m is the number of generations of self-pollination and n is the number of genes segregating. The porportion of completely homozygous plants under self-pollination is given in Fig. 6.2. It is clear that as the number of genes increases, the proportion of completely homozygous plants after a given number of generations of selfing decreases. But the effect of selfing is so strong that even if 100 genes

are segregating, more than 95 per cent of the population would be completely homozygous after only 12 generations. Linkage would increase the proportion of homozygous plants since it would reduce the number of genes segregating independently. Unequal survival of different genotypes would have the same effect as in the case of homozygosity.

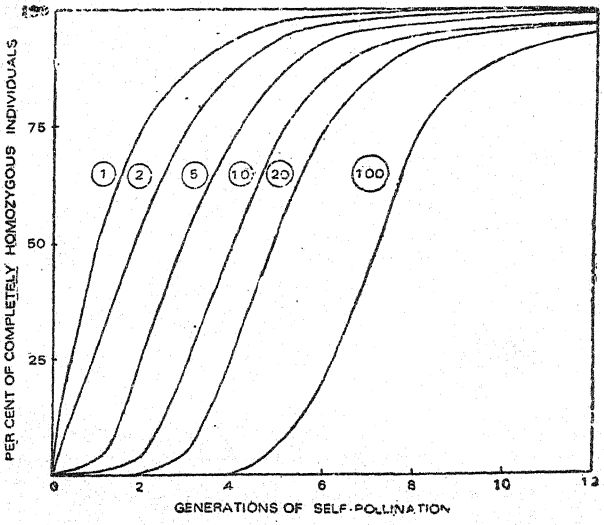


Fig. 6.2. Per cent of completely homozygous individuals after different generations of self-pollination ; the curves represent values when 1, 2, 5, 10, 20 and 100 genes are segregating.

Thus self-pollination has two main effects on the population : first, *all the plants in the population become completely homozygous, and,* second, *the population is a mixture of several homozygous genotypes.*

ORIGIN OF VARIATION IN PURELINES

It is generally accepted that purelines show genetic variation after some time. For example, barley variety Atlas was derived through single plant selection. Later, individual plant progenies were selected from Atlas. These progenies differed from each other with respect to quantitative as well as some minor qualitative characters. The variation in purelines may arise from machanical mixture, natural hybridization, chromosomal aberrations and mutations.

Mechanical Mixture. During cultivation, harvesting, threshing and storage, other genotypes may get mixed with a pureline. Such contaminations are quite common and may be avoided by careful handling. Often mass selection is practised to maintain the purity of purelines.

Natural Hybridization. In most self-pollinated species, a low amount of cross-pollination does occur, at least under some environmental conditions. Thus cross-pollination may occur with other genotypes grown nearby. Subsequent selfing of the hybrids would produce a number of new genotypes. In most self-pollinated species, natural hybridization can be avoided by isolating the pureline from other genotypes with a couple of rows of the same pureline, that is, two to three rows of the pureline on all the sides of the plot are rejected as border rows.

Chromosomal Aberrations. Varieties of several crops, *e.g.*, wheat, oats and barley etc., show a small frequency of meiotic anomalies, such as, chromatin bridges and chromosome fragments. These aberrations may lead to duplication and deficiency for small chromosome segments. Generally, the effects of small deletions and duplications would not be distinguishable from point mutations or gene mutations. It is, therefore, not definite as to what degree such aberrations are responsible for the origin of variation in purelines.

Mutation. *Mutation is a sudden heritable change, and generally denotes a chemical change in a gene, that is, point mutation or gene mutation.* In practice, however, it is difficult to distinguish point mutations from minute chromosomal aberrations, such as, duplication and deficiency. It may be generalised that almost ll the variability in plant species has originated due to gene mutations. Mutations occur spontaneously, they are random, recurrent and usually specific in their effect on phenotype.

Stadler in 1942 reported the spontaneous mutation rates for certain genes in maize. The mutation rates varied greatly from one gene to another. For example, gene *R* (produces colour) had a mutation rate of 4.92×10^{-4}, and *Su* (nonsugary endosperm) had a rate of 2.4×10^{-6}, while *Wx* (nonwaxy endosperm) did not show the mutant allelle (*wx*, waxy endosperm) in the 1,503,744 gametes tested. *Thus the spontaneous mutation rate for oligogenes may vary from one per 10,000 (i.e., 10^{-4}) to one per 1,000,000 (i.e., 10^{-6}) gametes.*

The frequency of mutations affecting quantitative characters is not known. This is primarily due to the difficulty in distinguishing the phenotypic effects of mutant allele from those of other genes and the environment. However, there is evidence to suggest that mutations affecting quantitative characters may be more frequent than those affecting qualitative characters.

In 1935 and 1936, East published his observations on some homozygous lines of *Nicotiana rustica.* These lines were obtained from plants that arose parthenogenetically during interspecific

hybridization. These lines were much more uniform than inbred lines produced by more than 10 generations of selfing. After four generations of self-pollination, the homozygyous lines became as variable as the inbreds. Mechanical mixture and natural hybrizadition were carefully avoided during the period of study. Therefore, the variation in these lines must have arisen from gene mutations. This suggests that mutations affecting quantitative characters are quite frequent, but generally they are difficult to detect.

One may then enquire about the possibility of improvement through spontaneous mutations occurring in purelines. The possibility of such an improvement is relatively small for two reasons. The *first* reason relates to the recurrence of mutations. Since mutations are recurrent, most of the mutations must have occurred in the past. Natural and artificial selection would have accumulated most of the superior alleles thus generated. Therefore, a new mutation is most likely to be inferior to the existing allele. The *second* reason arises from the frequency of mutations. The spontaneous mutation frequency is too low (10^{-5} to 10^{-6}) to create enough variability in few (3 or 4) generations to permit effective selection. But there are some instances of improvement of a pureline through spontaneous mutations, although of oligogenes. A spontaneous semidwarf mutant of a rice variety Kalimoonch 64 has been released for cultivation as a new variety Shyama. The dwarfing gene of Shyama is different from that of Dee-geo-woo-gen, the source of dwarfing gene in most of the semidwarf varieties of rice in India.

GENETIC ADVANCE UNDER SELECTION

Selection, of necessity, is made on the basis of phenotype, and phenotype is produced by the joint action of genotype and environment. Therefore, the phenotypic superiority of selected plants or families over the original population is not solely due to their genotypic superiority. *Improvement in the mean genotypic value of the selected families over the base population is known as genetic advance under selection.* Genetic advance under selection depends upon (1) the genetic variability among different plants or families in in the base population, (2) the heritability of the character under selection, and (3) the intensity of selection, *i.e.*, the proportion of plants or families selected. Genetic advance under selection may be calculated as follows.

$$Gs=(k)\ (\sigma p)\ (H)$$

where, Gs is genetic advance under selection, k is selection differential, σp is the phenotypic standard deviation of the base population (the population which is being subjected to selection), and H is the heritability of character under selection. The formula for genetic advance under selection may be rewritten as,

$$Gs=(k)\ (\sqrt{V_p})\ (V_g/V_p)$$

The selection differential, k, is based on the mean phenotypic values of the selected lines and of the base population, the pheno-

typic standard deviation (σ_p), and the intensity of selection, that is, the proportion of population selected. The k is expressed in terms of standard deviation units. Therefore, the value of k varies with the intensity of selection only (Table 6.2).

Table 6.2. Values of selection differential, k, for different selection intensities.

Selection intensity in per cent $(q/n)^* \times 100$	*Value of k*
1	2.64
2	2.42
5	2.06
10	1.76
20	1.40
30	1.16

*q is the number of selected plants or lines, and n is the total number of plants or lines in the original population.

The estimation of genetic advance under selection may be illustrated by using the data given in Table 4.6. The heritability is 0.914 and the phenotypic standard deviation is 1.92. Let us suppose that the intensity of selection is 5 per cent, that is, the extreme 5 per cent of the population will be saved to raise the next generation. The value of k, therefore, is 2.06. The genetic advance under selection would be,

$$Gs = (k)\,(\sigma_p)\,(H)$$
$$= 2.06 \times 1.92 \times 0.914 \text{ mg/plant}$$
$$= 3.6 \text{ mg nitrogen/plant}$$

It would be noted that the estimate of Gs has the same unit as the concerned mean. Gs may also be expressed as per cent of the population mean.

$$Gs \text{ (as per cent of population mean)} = (Gs/\bar{X}) \times 100$$

where, $\bar{X}$ is the mean of the base population.

EXPECTED GENETIC ADVANCE IN SEGREGATING POPULATIONS

The expected genetic gain as calculated by the foregoing formula is applicable to a mixture of purelines or clones, but it is not applicable to segregating generations. This is because purelines or clones give rise to progeny which are identical in genotype with the parent plant or family. Therefore, the genotypic value of progeny remains the same as that of the parent plant or family. But in segregating generations, the selected plants are likely to be heterozygous for few or several genes. Therefore, in the progeny new gene combinations would arise due to recombination. The genotypic value of new gene combinations is likely to differ from that of

the parent plant. This may be expected due to dominance and epistasic components of genetic variation, which depend on heterozygosity (except for the *i* component, *i.e.*, additive × additive interaction). Therefore, when dealing with segregating populations, heritability in narrow sense (*i.e.*, H=additive variance/phenotypic variance) is more appropriate for estimating *Gs*. The use of broad sense heritability estimates would give higher estimates of *Gs* than would be practically realised. In this case *Gs* may be estimated as follows.

$$Gs=(k)\ (\sigma p)\ (H, \text{narrow sense}).$$

Using the data on days to flowering in bajra (Chapter 4), we may estimate *Gs* as follows. The H (*ns*) is 0.394, and σp of the F_2, the segregating generation, is 4.79. Let us assume that 5 per cent of the extreme individuals will be saved. The *Gs* in this case would be,

$$Gs=(2.06)\ (4.79)\ (0.394) \text{ days}$$
$$=3.9 \text{ days}$$

SUMMARY

Selection consists of permitting the reproduction of some plants and stopping the others from reproducing. Selection appears to be as old as cultivation of plants. Vilmorin developed the progeny test. In *progeny test*, the value of a plant is judged from the performance of its progeny. Progeny test is the basis of modern plant breeding methods. In 1903, Johannsen put forward the pureline theory. A *punelline* is a progeny of a single, homozygous, self-pollinated plant. All the plants within a pureline have the same genotype. The variation among purelines has a genetic component, while that within a pureline is purely environmental. Genetic variation within a pureline may arise by mechanical mixture, natural hybridization, chromosomal aberrations and gene mutations. Mechanical mixtures and natural hybridization can be easily controlled. But mutations and chromosomal aberrations are beyond human control. Mutations affecting quantitative characters are known. The spontaneous mutation frequency for qualitative characters varies from 10^{-4} to 10^{-6}.

Self-pollination increases the frequency of homozygotes and the degree of homozygosity in the population. The population becomes homozygous rapidly, and finally it consists of a mixture of purelines. The decrease in heterozygosity for a single gene is by 50 per cent of that in the previous generation. The proportion of completely homozygous plants is given by $[(2^m-1)/2^m]^n$.

The difference between the genotypic values of the selected plants and of the original population is known as *genetic advance under selection*. In case of mixtures of purelines, the estimated genetic advance is a reliable estimate of actual genetic gain. But in the case of segregating populations, the estimates of genetic gain are often higher than the actual gains.

QUESTIONS

1. Write short notes on the following : (i) Vilmorin isolation principle, (ii) Pureline theory, and (iii) Genetic advance under selection.
2. Explain the pureline theory of Johannsen. Discuss the sources of genetic variation in purelines.
3. Explain genetic advance under selection. How does expected genetic advance under selection in a mixture of purelines differ from that in segregating populations ?

4. Give a brief account of the history of selection in self-pollinated populations. Describe progeny test and discuss its usefulness in plant breeding.

5. Discuss the effects of self-pollination on genotype and explain its implications in breeding of self-pollinated crops.

6. Briefly describe the contributions of the following scientists.
   (i) Vilmorin (ii) Johannsen
   (iii) Le Couteur (iv) Hallet

Suggested Further Reading

ALLARD, R.W. 1960. Principles of Plant Breeding. John Wiley and Sons, Inc., New York.

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CHAPTER 7

Hybridization : Techniques and Consequences

Natural variability present in self-pollinated populations is lost quickly when they are subjected to selection. Individual plant selection or pureline selection is the most common procedure used for the improvement of self-pollinated crops. Consequently, the variability is soon exhausted as the land varieties are replaced by purelines. For further improvement, therefore, new genetic variability has to be created by plant breeder. This is easily and most commonly achieved by crossing two different purelines.

The mating or crossing of two plants or lines of dissimilar genotype is known as hybridization. In plants, crossing is done by placing pollen grains from one genotype, the *male parent*, on to the stigma of flowers of the other genotype, the *female parent*. It is essential to prevent self-pollination as well as chance cross-pollination in the flowers of the female parent. At the same time, it must be ensured that the pollen from desired male parent reaches the stigma of female flowers for successful fertilization. The seeds as well as the progeny resulting from the hybridization are known as *hybrid* or F_1. The progeny of F_1, obtained by selfing or intermating of F_1 plants, and the subsequent generations are termed as *segregating generations*. The term *cross* is often used to denote the products of hybridization, *i.e.*, the F_1 as well as the segregating generations.

HISTORY OF HYBRIDIZATION

In comparison to selection, hybridization is of more recent origin. There is evidence that Babylonians and Assyrians hand-pollinated date plams as early as 700 B.C. for metaxenic effects of pollen. Clearly, the artificial pollination in this case was not for the purposes of crop improvement. *Metaxenia* is the effect of pollen on the maternal tissue of fruit. Sex in plants was discovered by

Camararious in 1694. In 1717, Thomas Fairchild produced the first artificial hybrid, the Fairchild's mule, by crossing sweetwilliam (*Dianthus barbatus*) with carnation (*Dianthus caryophyllus*). Subsequently, a large number of scientists used hybridization for scientific studies as well as for crop improvement. Notable among them are, Joseph Koelreuter who made many crosses in tobacco during 1760-1766, and emphasized hybrid vigour in F_1 ; Thomas Andew Knight who developed several varieties of apples, pears, peaches, grapes and currants during 1759-1835 ; and Goss, Sargaret, Gartner and Naudin had noted uniformity of F_1, dominance in F_1 and segregation and appearance of parental types in F_2. But it was left for Mendel (1865) to propose the clear-cut laws of inheritance. These and subsequent discoveries in genetics have given hybridization a scientific basis. Towards the end of nineteenth century, hybridization was widely used for crop improvement. With the help of genetic principles, the breeder is able to predict the progeny he is likely to obtain from a given cross. Therefore, he can plan the crosses from which he is most likely to obtain the desired plant type. Today, hybridization is the most common method of crop improvement, and the vast majority of crop varieties have resulted from hybridization.

OBJECTIVES OF HYBRIDIZATION

The chief objective of hybridization is to create genetic variation. When two genotypically different plants are crossed, the genes from both the parents are brought together in F_1. Segregation and recombination produce many new gene combinations in F_2 and the later generations, *i.e.*, the segregating generations.. The degree of variation produced in the segregating generations would, therefore, depend on the number of heterozygous genes in the F_1. This will, in turn, depend upon the number of the genes for which the two parents differ. If the two parents are closely related, they are likely to differ for a few genes only. But if they are not related, or are distantly related, they may differ for several, even a few hundred, genes. However, it is not likely that the two parents will ever differ for all the genes. Therefore, when it is said that the F_1 is 100 per cent heterozygous, it has reference only to those genes for which the two parents differ.

The aim of hybridization may be transfer of one or few qualitative characters, improvement of one or more quantitative characters, or use of the F_1 as a hybrid variety. These objectives are briefly discussed below.

Combination Breeding. *The main aim of combination breeding is the transfer of one or more characters into a single variety from other varieties.* These characters may be governed by oligogenes or polygenes. The intensity of the character in the new variety is either comparable to or, more generally, lower than that in the parent variety from which it was transferred. In this approach, increase in the yield of a variety is obtained by correcting the weaknesses in the

yield contributing traits, *e.g.*, tiller number, grains per spike, test weight etc., of the concerned variety. A familiar example of combination breeding is that for disease resistance. The backcross method of breeding was designed for combination breeding, and often pedigree method also fulfils the same purpose. In combination breeding, the genetic divergence between parents is not the major consideration. What is important is that one of the parents must have in a sufficient intensity the character(s) under transfer, while the other parent is generally a popular variety.

Transgressive Breeding. *Transgressive breeding aims at improving yield or its contributing characters through transgressive segregation. Transgressive segregation* is the production of plants in an F_2 generation that are superior to both the parents for one or more characters. Such plants are produced by an accumulation of plus or fovourable genes from both the parents as a conseqence of recombination. Obviously, the parents involved in hybridization must combine well with each other, and should preferably be genetically diverse, *i.e.*, quite different. This way, each parent is expected to contribute different plus genes which when brought together by recombination give rise to transgressive segregants. As a result, the intensity of character in the transgressive segregant, *i.e.*, the new variety, is greater than that in either of the parents. The pedigree method of breeding and its modifications, particularly the population approach, are designed for the production of transgressive segregants.

Hybrid Varieties. In most self-pollinated crops, F_1 is more vigorous and higher yielding than the parents. Wherever it is commercially feasible, F_1 may be used directly as a variety. In such cases, it is important that the two parents should produce an outstanding F_1.

TYPES OF HYBRIDIZATION

The plants or lines involved in hybrdization may belong to the same variety, different varieties of the same species, different species of the same genus or species from different genera. Based on the taxonomic relationsphips of the two parents, hybridization may be classified into two broad groups : (1) intervarietal and (2) distant hybridzation.

Intervarietal Hybridization. *The parents involved in hybridization belong to the same species ; they may be two strains, varieties or races of the same species.* It is also known as *intraspecific hybridization.* In crop improvement programmes, intervarietal hybridization is the most commonly used. In fact, it is so common that it may often appear to be the only form of hybridizasion used in crop improvement. An example would be crossing of two varieties of wheat (*T. aestium*), rice (*O. sativa*) or some other crop. The intervarietal crosses may be simple or complex depending upon the number of parents involved.

1. **Simple Cross.** *In a simple cross, two parents are crossed to produce the* F_1. The F_1 is selfed to produce F_2 or is used in a backcross programme, *e.g.*, $A \times B \longrightarrow F_1 (A \times B)$

2. **Complex Cross.** *More than two parents are crossed to produce the hybrid,* which is then used to produce F_2 or is used in a backcross. Such a cross is also known as *convergent cross* because this crossing programme aims at converging, *i.e.*, bringing together, genes from several parents into a single hybrid. A few examples of convergent cross are described in Fig. 7.1. As crop improvement progresses, the crop varieties would accumulate more and more favourable genes. This would lead to greater similarities between even unrelated varieties. In view of this, it may be expected that in future complex crosses would become more and more important. In breeding of highly improved self-pollinated crops like wheat and rice, complex crosses are a common practice today. Complex crosses would become routine in near future in the improvement of other self-pollinated crops with the progress in the level of their improvement.

Distant Hybridization. *Distant hybridization includes crosses between different species of the same genus or of different genera.* When two species of the same genus are crossed, it is known as *interspecific hybridization* ; but when they belong to two different genera, it is termed as *intergeneric hybridization.* Generally, the objective of such crosses is to transfer one or few simply inherited characters like disease resistance to a crop species. Sometimes, interspecific hybridization may be used for developinng a new variety, *e.g.*, Clinton oat variety was developed from a cross between *Avena sativa* × *A. byzantina* (both hexaploid oat species), and CO 31 rice variety was developed from the cross *Oryza sativa* var. *indica* × *O. perennis.* Almost all the present-day sugarcance varieties have been developed from complex crosses between *Saccharum officinarum* (noble canes), *S. barberi* (Indian cones) and other *Saccharum* species, *e.g.*, *S. spontaneum* (Kans.). The improvement in fiber length of Indian cotton (*Gossypium arboreum*) has been brought about by crossing it with American cultivated cotton (*G. hirsutum*). Many improved varieties have resulted from such crosses. Intergeneric hybridization may also be used to develop a new crop species, *e.g.*, *Triticale* from a cross between *Triticum* sp. and *Secale cereale* (rye). Wild species often provide genes which are not present in the cultivated species. For example, many of the genes for rust resistanee in wheat are derived from related wild species. Distant hybridization is likely to become increasingly important in the correction of specific defects of crop species. In many cases, wild species may contribute valuable 'yield genes' as well to the cultivated species.

THE HYBRIDIZATION PROGRAMME

Hybridization is the most important method of crop improvement. The process of hybridization itself is fairly simple and easy.

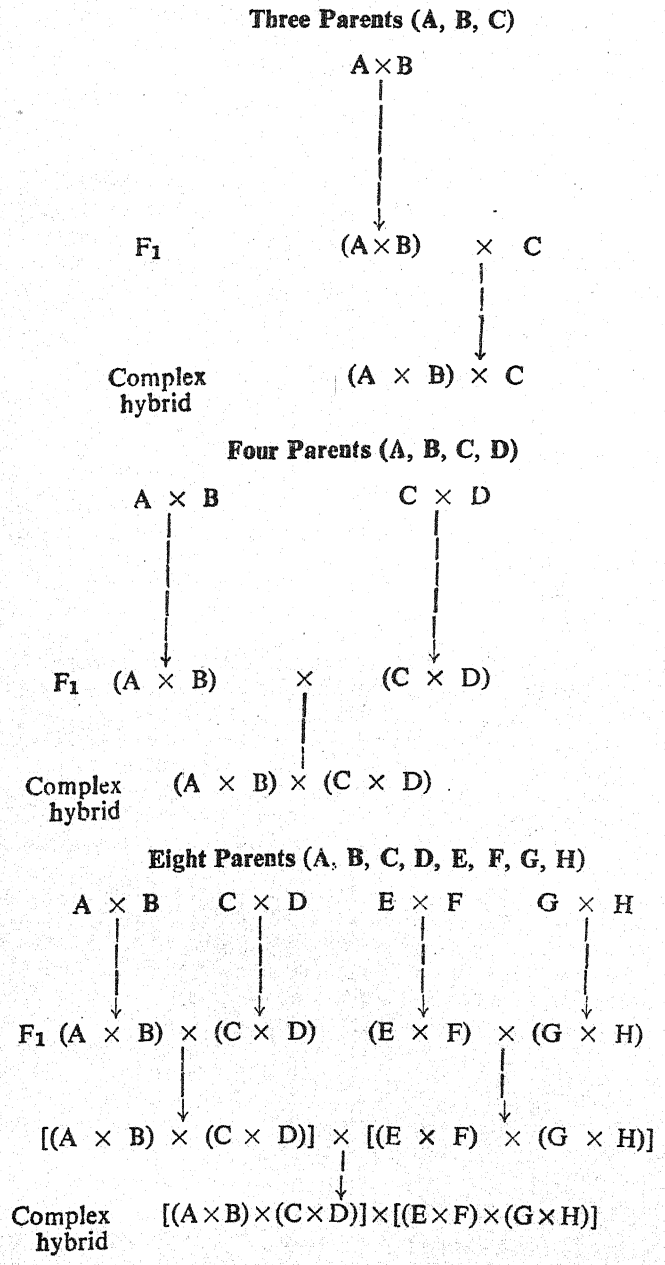


Fig. 7.1. Complex crosses involving 3, 4 and 8 parents.

Consequently, a beginner may be tempted to make many crosses. But the difficulty in using hybridization in crop improvement lies in the handling of segregating generations. The problems that a breeder has to face are given below.

Raising the Segregating Generations. Generally, F_2 and later generations consist of several thousand plants. Raising of F_2 from several crosses at the same time requires money, labour, land and other facilities. Often these facilities are but limited.

Handing of the Segregating Generations. Selection for desirable plant types has to be made in F_2 and subsequent generations. Selection for qualitative characters is simple and quick, but selection for quantitative characters is often difficult and time-consuming. If the breeder desires to practice selection based on scientific considerations, he will be able to handle only few crosses at a time.

These two considerations put a limit on the number of crosses the breeder can handle at a time. Thus a breeder should not make too many crosses only to discard most of them at a later stage due to the lack of facilities.

Advance Planning. This problem is more important than the first two, but is less obvious. The development of a new variety usually takes 7-8 years. Therefore, a breeder has to foresee the requirements about 8-9 years in advance. He should be able to plan his breeding programme according to these requirements so that he has a veriety ready when the need arises. Thus he must know the requirements of the market in terms of quality etc., the diseases and pests of the area, the environmental fluctuations and the prevalent management practices among the farmers. This he must know for the present time, and should also be able to expect and forecast for the future as well. This means that the breeder must be thoroughly familiar with all the aspects of the crop species he is working with. Further, he must clearly define the type of variety he wants to produce, the important characters he wishes to improve. Then he has to search for suitable parents which will contribute these characters, and use them in a suitable hybridization programme. For this the breeder must be familiar with the existing varieties, and he should be able to obtain information on the characteristics of the germplasm collections and the wild relatives of the crop species.

In conclusion, the breeder should have well-defined, clear-cut objectives based on the present and the expected future needs in developing a new variety. He should select the parents accordingly and use them in a suitable hybridization programme.

THE PROCEDURE OF HYBIDIZATION

Once the breeder has decided the objectives of programme, he is ready to begin hybridization. There are seven steps involved in hybridization : (1) choice of parents, (2) evaluation of parents,

(3) emasculation, (4) bagging, (5) tagging, (6) pollination, and (7) harvesting and storage of F_1 seed.

Choice of Parents. The choice of parents mainly depends upon the objective of breeding programme. In addition to other objectives, increased yields are always the objective of a breeder. Therefore, at least one of the parents involved in a cross should be a well adapted and proven variety in the area for which the new variety is being developed. The other variety should be having the characters that are absent in this variety. In combination breeding, the genetic diversity of the parents is not important, but in transgressive breeding genetic diversity is of great importance. For transgressive breeding, it should be made sure that the parents differ for many genes affecting yield or some other character of importance. However, it would be desirable that the performance of parents is good and that they are well adapted in the areas where they are commonly grown. Further, some parents produce superior F_1s and F_2s, while others do not. This property of the parents is known as *combining ability* and can be estimated by one of the many biometrical techniques. Combining ability of the parents may serve as a useful guide in the selection of parents for a hybridization programme.

It is essential that all the characters sought to be improved are present in one or the other parent. If necessary, three or more parents may be included in a complex cross. One should remember that a character absent in the original parents will generally not appear in the segregating generations, except for the possibility of transgressive segregations and some gene interactions, *e.g.*, complementary gene action. Thus the choice of parents is the basic step in a hybridization programme and often, more than anything else, determines its success or failure.

Evaluation of Parents. If the performance of parents in the area where breeding is to be done is known, evaluation is not necessary. But if their performance in the area is not known, it should be determined, particularly for the characters they are expected to contribute and for disease resistance. Disease reaction is important because an introduced parent may be susceptible to the new races of the pathogen occurring in the area, or even new diseases present in the area for which their reaction may not be known. New strains should also be checked for mechanical mixture, and for heterozygosity if the crop species shows about 5 per cent cross-pollination. In the latter case, it may be necessary to self-pollinate the parent for one or more generations if it is suspected to be heterozygous.

Emasculation. *The removal of stamens or anthers or the killing of pollen grains of a flower without affecting in any way the female reproductive organs is known as emasculation.* The purpose of emasculation is to prevent self-fertilization in the flowers of female parent. In dioecious plants, male plants are removed, while in monoecious species the male flowers, *e.g.*, in castor, or the male inflorescence, *e.g.*, in maize, are removed to prevent self-pollination. But emasculation is essential in bisexual flowers. It may be done in

any one of several ways. The method suitable for a species is largely determined by the size of its flowers, the amount of seed needed the number of seeds per fruit and the purpose for which the hybrid seeds are required. In species with relatively large flowers, hand emasculation may be adequate in most hybridization programmes. On the other hand, in species with small flowers hand emasculation is generally difficult, tiring and time consuming. Similarly, when large quantities of hybrid seed are needed, hand emasculation is impractical in most self-pollinated crop species, except in those that set many seeds in one fruit, *e.g.*, tobacco (about 2000 seeds per fruit), tomato, brinjal etc. Similarly, for accurate genetic studies, hand emasculation is desirable because with other methods there may be some self-pollination.

The efficiency of an emasculation technique may be tested by bagging the emasculated flowers without pollination. The amount of seed thus set would indicate the frequency of chance self-fertilization during emasculatian. If the seeds are to be used in genetic studies, there should be no self-pollination during emasculation. In most of the crop improvement programmes and in the production of hybrid seed, a small amount of self pollination may be permissible. But it is desirable that self-pollination should be kept to the minimum, preferably to the zero level. The various techniques of emasculation are outlined below.

Hand Emasculation. In species with relatively large flowers, stamens or anthers are removed with the help of forceps. The exact details of the procedure vary from one crop species to the other. Emasculation is done before the anthers are mature and the stigma has become receptive to minimise accidental self-pollination. Usually, stigma receptivity is at its peak during the morning hours when the flowers open, but different crop species show considerable variation in the duration for which their stigma remains highly receptive. Thus emasculation is generally done in the evening, between 4 and 6 P.M., one day before the anthers are expected to dehisce or mature and the stigma is likely to become fully receptive. Therefore, the flowers selected for emasculation are likely to open the next morning. With some experience, the breeder should be able to select such flowers without much difficulty. Generally, it is desirable to remove the older and the younger flowers located close to the flower to be emasculated in order to avoid confusion in identification of crossed pods etc.

A generalised procedure for hand emasculation is as follows. The corolla of the selected flowers is opened and the anthers are carefully removed with the help of fine-tip forceps. In many crop species, the androecium is epipetalous, *e.g.*, in cotton, jute, brinjal, sweet potato, tomato, potato, bhindi, etc. In such cases, sometimes the corolla may be totally removed alongwith the epipetalous stamens. In cereals, one-third of the empty glumes may be clipped off with scissors to expose the anthers. In wheat (*T. aestivum*) and oats (*A. sativa*), only two large florets per spikelet are left ; the other florets

are removed. *Care must be taken to remove all the anthers from the flowers without breaking them and, the most important, the gynoecium must not be injured.* The first precaution in necessary to prevent self-pollination, while the latter is necessary for seed set after the desired pollination. Further, it is desirable to cause the minimum damage to the flower during emasculation. *An efficient emasculation technique should prevent self-pollination and produce a high percentage of seed set on cross-pollination.*

The technique of emasculation in barley (*H. vulgare*) is described in some detail. A spike that is about to emerge from the flag leaf would be generally appropriate for emasculation. The flag leaf is carefully peeled away, but not removed, to expose the spike. The spikelets from the lower and the upper 2-3 nodes are clipped off. On the remaining nodes, generally the two side spikelets are removed leaving the central spikelet only. This is done because the stigmas of central and side spikelets become receptive at different times. The top one-third of ma and palea is clipped off ; the three anthers present in each spikelet are now easily accessible and are removed with the help of forceps. Care must be taken not to damage the stigma and ovary, and to remove all the anthers from all the spikelets.

Suction Method. This method is useful in species with small flowers. Emasculation is done in the morning just before or immediately after the flowers open. The petals are generally removed with forceps exposing the anthers and the stigma. A thin rubber or glass tube attached to a suction hose is used to suck the anthers from the flowers. The tube is also passed over the stigmas to suck any pollen grains present on their surface. The suction may be produced by an aspirator attached to a water tap, or by a small suction pump. The amount of suction used is very important. The suction should be enough to suck the stamens and pollen grains, but not the flowers or the gynoecium. *With suction method, considerable self-pollination (up to 15 per cent) is likely to occur.* Washing the stigma with a jet of water may help in reducing self-pollination. However, self-pollination cannot be eliminated in this method.

Hot Water Emasculation. Pollen grains are more sensitive than the female reproductive organs to both genetic and environmental factors. This property is utilized to kill the pollen grains with hot-water or other agents like alcohol treatment or cold water treatment without damaging the female reproductive organs. In the case of hot water emasculation, the temperature of water and the duration of treatment vary from crop to crop, and must be determined for every species. For jowar (*S. bicolor*), treatment with water at 42-48°C for ten minutes is found to be suitable. In case of rice (*O. saiva*), 10 minute treatment with water at 40-44°C is adequate. Hot water treatment is given before anther dehiscence and prior to opening of flowers. The hot water is generally carried in thermos flasks and the whole spike is immersed in the water. Emasculation with hot water

is generally highly effective in killing all the pollen grains provided the correct temperature and treatment duration are used.

Alcohol Treatment. It is not a commonly used method of emasculation. The method consists of immersing the flower or the inflorescence in alcohol of a suitable concentration for a brief period, followed by rinsing with water. In sweet clover, immersion of the inflorescence in 57 per cent alcohol for 10 seconds was highly effective ; the percentage of selfing was only 0.89. It is a better method of emasculation than the suction method. However, the duration of treatment is of utmost importance. Even a slightly prolonged period of treatment, say a few seconds more than the recommended, would greatly reduce seed set. This is because the female reproductive organs would also be killed by a longer treatment.

Cold Treatment. Cold treament, like hot water treatment, kills pollen grains without damaging gynoecium. In case of rice, treatment with cold water at 0-6°C kills pollen grains without affecting gynoecium. Keeping wheat plants at 0-2°C for 15-24 hours kills the pollen grains. Cold treatment is less effective than hot water treatment. The amount of self-pollination is generally greater in cold treatment than in the case of hot water treatment.

Genetic Emasculation. Genetic or cytoplasmic male sterility may be used to eliminate the necessity of emasculation (Chapter 3). Many species are self-incompatible. In such cases, emasculation is not necessary because self-fertilization will not take place. In certain genotypes and under certain environments, the male sterility and self-incompatibility systems may breakdown partially. However, for commercial hybrid seed production, male sterility is the most feasible method of emasculation. Protogyny facilitates crossing without emasculation. Since the stigmas become receptive before the anthers mature, hand pollination ensures seed set from cross-pollination and prevents self-fertilization, *i.e.*, in bajra (*P. americanum*).

Bagging. Immediately after emasculation, the flowers or the inflorescences are enclosed in suitable bags of appropriate size to prevent random cross-pollination. In cross-pollinated crops, like maize, the male flowers are also bagged to maintain the purity of pollen used for pollination. The bags may be made of paper, butter paper, glassine or fine cloth. Butter paper or vegetable parchment bags are the most commonly used. Cloth bags are generally not preferred since they permit some degree of chance cross-pollination. The bags are tied to the base of inflorescence or to the stalk of flower with the help of thread, wire or pins designed for the purpose. The moisture and temperature are generally higher inside the bags as compared to the outside. Therefore, bagging may promote fungus development on the fruit or the spike. This may be prevented by removing the bags after the danger of cross-pollination is over usually 2-3 days after pollination.

Tagging. The emasculated flowers are tagged just after bagging. Tags are available in different sizes. In most of the crops, circular

tags of about 3 cm diameter, or rectangular tags of 3 × 2 cm are used. In crops like maize (*Z. mays*), bajra (*P. americanum*) and jowar (*S. bicolor*), bigger tags (6 × 3 cm) are used. The tags are attached to the flower or the inflorescence with the help of thread. The following information is recorded on the tags with a carbon pencil.

1. Date of emasculation
2. Date of pollination
3. Names of the female and the male parents. The name of the female parent is written first, and that of the male parent is written later, *e.g.*, A × B denotes that A is the female parent and B is the male parent.

Pollination. The two most important operations that determine the amount of seed set in hybridization are emasculation and pollination. During emasculation, damage to the female reproductive organs must be avoided. *In case of pollination, mature, fertile and viable pollen should be placed on a receptive stigma to bring about fertilization.* The duration of pollen viability after anther dehiscence varies greatly from one species to another, *e.g.*, a few minutes in wheat and oats to a few hours in maize. Therefore, it is advisable that fresh pollen from mature anthers should be used for pollination. The time of anther dehiscence falls within the duration of stigma receptivity and both generally coincide with the opening of flowers. Anthers generally dehisce during morning ; the exact time varies with the species.

The pollination procedure consists of collecting pollen from freshly dehisced anthers of the male parent and dusting this pollen on the stigma of emasculated flowers. This may be done in one of the following several ways.

(i) Pollen grains are collected in a bag, and are used for dusting stigmata of female inflorescence or of emasculated flowers, the *e.g.*, in maize, bajra etc.

(ii) Mature anthers are collected from the flowers of male parent. The pollen is liberated and applied to the stigma with the help of a camel hair brush, pieces of paper, tooth pick or forceps.

(iii) Anthers are collected and allowed to burst directly over the stigma. In rice, oats, wheat and barley, one anther is generally inserted in each floret where it dehisces and covers the stigma with pollen grains.

(iv) The spike of male inflorescence is shaken over the emasculated inflorescence just when the anthers are about to dehisce. As a result, the exposed stigma are covered with pollen. This is commonly done in wheat and barley where the lemma and palea are clipped off to expose the stigma of emasculated flowers. The lemma and palea of the spike of male parent are also clipped of to expose the anthers, which are used as the source of pollen.

(v) In species like maize, the male inflorscence may be detached and enclosed in the bag covering the female inflorescence. In case of

bajra and jowar, panicles from the male parent may be enclosed in the same bags that enclose the panicles of female parent.

Harvesting and Storing the F_1 Seeds. The crossed heads or pods should be harvested and threshed. The seeds should be dried and properly stored to protect them from storage pests. Proper care should be taken to avoid contamination of the hybrid seed with other seeds. The seeds from each cross should be kept separately and, preferably, the seeds should be kept alongwith the original tags.

RAISING THE F_1 GENERATION

If the parents involved in a cross differ for genes affecting seedling characteristics, the parent with the dominant character should be used as the male parent. This would allow the identification of selfed seeds in the F_1 generation. The breeder should take advantage of such marker genes if they are available in the cross he has planned.

There is some controversy regarding the size of F_1. Allard feels that even 12 F_1 seeds are enough for most breeding progammes, except the backcross. But Elliot is of the opinion that the F_1 population should be as large as possible, because F_1 is the only generation where all the genes for which the two parents differ would be in the heterozygous condition. Thus the larger the size of F_1, the greater the opportunity of rare recombinations to occur. Generally, a larger F_1 size is not used because (1) hand crossing is tedious and time taking, (2) with a large F_1 population, the F_2 becomes unmanageable, (3) many breeders feel that the size of F_1 is not important, and (4) rare recomtinations may not be detected due to the masking influence of environment. But wherever possible, a larger F_1 population is more desirable than a smaller one. The F_1 is usually allowed to self-pollinate, but in a backcross programme it is crossed to one of the parents.

SELFING

The objective of selfing is to avoid cross-pollination and to ensure self-pollination. The technique of selfing varies from one crop to the other depending upon the mode of reproduction. In self-pollinated crops, selfing is the natural mode of reproduction, and to ensure selfing no operation is needed. But in the case of often cross-pollinated species, the flowers are generally bagged to prevent cross-pollination. In case of cross-pollinated species with bisexual flowers or with both male and female flowers in a single inflorescence, bagging the entire inflorescence, or sometimes the whole plant is adequate. The bags may be shaken daily to help pollen dissemination and pollination. In legumes like alfalfa (*M. sativa*), hand tripping of flower is essential for self-fertilization since the stigma has a waxy covering which must be removed to make it receptive. In a crop like maize, the male and female inflorescences are bagged ; the pollen is collected in the tassel bag and dusted on the silk of the female

inflorescence. Alternatively, the tassel may be cut and enclosed in the bag covering cob. The cut end of tassel may be kept in water contained in a small bottle to keep the tassel alive for a longer period.

DIFFICULTIES IN HYBRIDIZATION

Ordinarily intervarietal hybridization presents little problem, if any, while distant hybridization is often beset by many difficulties. In many crops, however, some varieties, particularly when used as the female parent, show a considerably lower seed set than other varieties. This problem is not a serious one, and can be easily resolved by pollinating a proportionately larger number of flower buds.

A more serious problem of hybrid (F_1) mortality due to lethal genes is encountered in several cross combinations of some crops. An excellent example is furnished by wheat (*Triticum aestivum*) where F_1 plants from many cross combinations show hybrid necrosis. Leaves of necrotic plants show discolouration and drying of leaves which begins at the tips of older leaves and progresses towards their base (leaf sheath). The affected areas of leaves first turn darker green, then greyish brown as the chlorophyll degenerates and finally the leaf tissues die. The hybrid necrosis in wheat is believed to be the result of two dominant complementary genes Ne_1 and Ne_2 located on the chromosomes $5BL$ and $2B^S$, respectively. According to an estimate, about 18% of wheat varieties possess Ne_1, while more then 42% of the varieties have Ne_2.

There is a considerable variation in the severity of hybrid necrosis in different cross combinations of wheat. In severe cases, necrosis may develop in the first or second leaf stage and the plants may die by the 3-6 leaf stage. In mild cases of necrosis, only leaf tips may be affected and the plants may grow and reproduce normally. All the grades between these two extremes are known to occur and a 0-8 scale has often been used for scoring the intensity of necrosis. This variation in the intensity of necrosis is presumably due to multiple alleles of both Ne_1 ($Ne_1{}^w$, $Ne_1{}^m$ and $Ne_1{}^s$ and Ne_2 ($Ne_2{}^w$, $Ne_2{}^m$, $Ne_2{}^{ms}$ and $Ne_2{}^s$) genes which have weak (w), moderate (m), moderately strong (ms) or strong (s) necrotic effects.

The problem of hybrid necrosis (or other lethal genes) may be overcome by (i) keeping necrotic crosses out of the breeding programme ; (ii) using one parent having recessive alleles of both the *Ne* genes ; (iii) inducing mutation for the recessive allele of the concerned *Ne* gene ; (iv) utilizing spontaneous mutants having the recessive allele of the concerned *Ne* gene, where available ; (v) using a chemical treatment, *e.g.*, spray of an amino acid, such as, proline, in the case of wheat ; and (vi) growing the F_1 plants in an environment, if known, which will prevent the expression of the lethal gene(s), *e.g.*, termperatures over 22°C in the case of *Ne* genes of wheat.

CONSEQUENCES OF HYBRIDIZATION

In self-pollinated species, it is the easiest to permit self-pollination in F_1 to produce an F_2 generation. Segregation and recombination of genes would produce several new genotypes, in addition to the two parental types, in F_2. The number of genotypes produced in F_2 increases rapidly as the number of segregating genes increases (Table 6.1). The number of phenotypes with and without dominance also increases rapidly with an increase in the number of segregating genes. If, for example, 10 genes are segregating, the F_1 would produce 1,024 types of gametes, 59,049 different genotypes in F_2, and 1,024 phenotypes with full dominance and 59,049 phenotypes without full dominance and epistasis. The smallest size of a perfect F_2 population (that includes at least one plant of every genotype) would be 1,084,576. Most of the intervarietal crosses would differ for many more genes. It is, therefore, impractical to raise a perfect F_2 population for any cross. Thus the difficulty in recovery of rare recombinants from segregating generations can be easily appreciated. This difficulty would be great enough even if the phenotypic effects of the genes were easily recognisable. However, in the case of quantitative characters the effects of genes are masked by those of the environment and gene interactions making the identification of rare recombinants even more difficult.

In F_2, a vast majority of plants would be heterozygous for one or more genes. Heterozygosity reduces the effectiveness of selection because such plants segregate to produce variable progeny, *i.e.*, the genetic value of progeny is not the same as that of the parent plants. The frequency of completely homozygous plants in any segregating generation is given by the following formula.

Proportion of completely homozygous plants $=[(2^m-1)/2^m]^n$

where m is the number of generations of self-pollination and n is the number of genes segregating. As the number of generations of self-pollination increases, the frequency of completely homozygous plants increases sharply (Table 7.2). Consequently, the confusing effects of heterozygosity are reduced and selection becomes more effective in the advanced selfed generations. Many breeders feel that selection on individual plant basis should be delayed till F_5 or F_6 generation.

These considerations have assumed equal survival of all the genotypes, and no linkage. There is evidence that heterozygotes may be favoured in nature. The increase in homozygosity, therefore, would be less than theoretically expected. Linkage would decrease the frequency of recombinant types and increase the frequeney of completely homozygous plants. The latter effect is produced because linkage reduces the number of genes segregating independently. Linkage between favourable genes makes selection easier because the two desirable characters tend to be inherited together. But linkage between favourable and unfavourable genes is a problem because larger populations would be required to break this linkage.

Table 7.1. The number of different types of gametes produced by F_1 and the composition of F_2 generation from crosses segregating for different number of genes.

Number of genes segregating	*Types of gametes produced by* F_1	*Number of different genotypes in* F_2	*Smallest perfect* F_2 *population*	*Number of phenotypes in* F_2		*Number of completely homozygous genotypes*
				Full dominance	*No dominance*	
1	2	3	4	2	3	2
2	4	9	16	4	9	4
3	8	27	64	8	27	8
4	16	81	256	16	81	16
5	32	243	1,024	32	243	32
8	256	6,561	65,536	256	6,561	256
10	1,024	59,049	1,048,576	1,024	59,049	1,024
15	32,768	14,348,907	1,073,741,824	32,768	14,348,907	32,768
20	1,048.576	3,486,784,401	1,099,511,627,776	1,048,576	3,486,784,401	1,048,576
n	2^n	3^n	4^n	2^n	3^n	2^n

+This is also the number of genotypes produced in a back-cross and the smallest perfect back-cross population.

Table 7.2. Frequency of completely homozygous plants in F_2 and the subsequent generations of crosses segregating for different number of genes.

Number of genes segregating	*Frequency (in per cent) of completely homozygous plants*				
	F_2	F_3	F_4	F_5	F_7
1	50.0	75.0	87.5	93.8	98.4
2	25.0	56.3	76.6	87.9	96.9
3	12.5	42.2	67.0	82.4	95.4
4	6.3	31.7	58.6	77.2	93.9
5	3.1	23.7	51.3	72.4	92.4
10	0.097	5.6	26.3	52.5	85.4
20	0.00009	0.32	6.9	27.5	73.0

* The number of generations of self-pollination will be 1 in F_2, 2 in F_3, 3 in F_4 and n-1 in Fn generation since F_1 is the result of hybridization and only F_2 and the subsequent generations are due to selfing.

In case of quantitative characters, recombination may produce genotypes superior to the two parents. For example, if a genotype *AA bb CC dd* is crossed with another variety with the genotype *aa BB cc DD*, the F_1 would be *Aa Bb Cc Dd*, and in F_2 plants with genotypes *AA BB CC DD* and *aa bb cc dd* would be produced. These plants would be superior and inferior, respectively, to both the parents. Such recombinants are known as *transgressive segregants*. Breeding for yield generally aims at the recovery of transgressive segregants. But if one considers the frequency of such segregants in F_2, which is 2 out of 4^n plants (where n is the number of genes segregating), it would be apparent that the chances of recovery of such segregants are indeed very low. Most of the plants would, however, have some good genes from each parent, but rarely a plant in a small F_2 population would have all the good genes from both the parents.

SUMMARY

Hybridization consists of crossing of two plants or lines with different genotypes. The purpose of hybridization is to bring together in F_1 genes from the two parents for the purpose of *combination* or *transgressive breeding*. The F_1 may be used as a variety where it is commercially feasible. The two parents involved in a cross may belong to the same variety, different varieties of the same species (*intervarietal* or *intraspecific hybridization*), or to different species of the same genus or to different genera (*distant hybridization*). The intervarietal hybridization may involve two (*simple cross*) or more parents (*complex cross*). The function of distant hybridization generally is to transfer specific characters to a cultivated species.

The breeder should have clear-cut objectives in making a cross, and the parents should be selected to fulfil these objectives. The parents are evaluated for various characteristics before being crossed. The flowers of parent to be used as female are emasculated by hand, suction, hot, cold or alcohol treatment, male sterility or self-incompatibility. The emasculated flowers are immediately bagged and tagged. Emasculation is done one day before the

stigma becomes receptive, usually in the evening between 4-6 P.M. The emasculated flowers are pollinated by hand the next morning. It is desirable to use as large an F_1 population as the resources permit to provide the maximum chance for recombination.

Segregation and recombination produce a large number of genotypes in F_2. The number of different genotypes possible in F_2 increases geometrically with an increase in the number of segregating genes. Homozygosity increases rapidly with continued selfing. The frequency of completely homozygous plants also increases rapidly. By F_7, about 73 per cent of the plants become completely homozygous when 20 genes are segregating. Transgressive segregation may occur, but usually the recovery of such recombinants will be very difficult.

QUESTIONS

1. What is hyridization ? Briefly describe the different types of hybridization. Discuss in detail the objectives of hybridization.
2. Describe in brief the various steps involved in hybridization.
3. Comment on the following : (i) Parents involved in hybridization should be diverse, (ii) Linkage increases the frequency of completely homozygous plants as well as homozygosity, (iii) Transgressive segregation in plant breeding, and (iv) Perfect F_2 population for the recovery of all gene combinations from an intervarietal hybrid.
4. Define emasculation. Briefly describe the various methods of emasculation.
5. Write short notes on the following. (i) Pollination, (ii) Genetic emasculation, (iii) Bagging, (iv) Hot water emasculation, (v) Selection of parents for a hybridization programme, (vi) Complex crosses, (vii) Combination breeding, and (viii) Transgressive breeding.

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CHAPTER 8

Genetic Composition of Cross-Pollinated Populations

Cross-pollinated crops are highly heterozygous due to free intermating among their plants. They are often referred to as *random mating* populations because each individual of the population has equal opportunity of mating with any other individual of that population. Such a population is also known as *Mendelian population* or *panmictic population.* A Mendelian population may be thought of having a *gene pool* consisting of all the gametes produced by the population. Thus the gene pool may be defined as the sum total of all the genes present in a population. Each generation of a Mendelian population may be considered to arise from a random sample of gametes from the gene pool of previous generation. For this reason, it is not possible to follow the inheritance of a gene in a Mendelian population by using the techniques of classical genetics. To understand the genetic make-up of such populations a sophisticated field of study, *population genetics*, has been developed. We shall examine the elementary principles of population genetics in order to understand the genetic composition of random mating populations.

THE HARDY-WEINBERG LAW

The Hardy-Weinberg law is the fundamental law of population genetics and provides the basis for studying Mendelian populations. This law was independently developed by Hardy (1908) in England and Weinberg (1909) in Germany. *The Hardy-Weinberg law states that the gene and genotype frequencies in a Mendelian population remain constant generation after generation if there is no selection, mutation, migration or random drift.* The frequencies of the three genotypes for a locus with two alleles, say A and a, would be p^2 AA, $2pq$ Aa, and q^2 aa ; p represents the frequency of A and q represents the frequency of a allele in the population. The sum of p and q is one, *i.e.*, $p+q=1$. Such a population would be at equilibrium since the genotypic frequencies would be stable, that is,

would not change, from one generation to the next. This equilibrium is known as *Hardy-Weinberg equilibrium.* A population is said to be at equilibrium when frequencies of the three genotypes, *AA*, *Aa* and *aa* are p^2, $2pq$ and q^2, respectively. Whether a population is at equilibrium or not can be easily determined by a chi-square test.

Hardy-Weinberg law can be easily explained with the help of an example. Let us consider a single gene with two alleles, *A* and *a*, in a random mating population. There would be three genotypes *AA*, *Aa* and *aa*. Suppose the population has *N* individuals of which *D* individuals are *AA*, *H* individuals are *Aa* and *R* individuals are *aa* so that $D+H+R=N$. The total number of alleles at this locus in the population would be $2N$ since each individual has two alleles at a single locus. The total number of *A* alleles would be $2D+H$ because *AA* individuals have two *A* alleles each, while each *Aa* individual has only one *A* allele. The ratio $(2D+H)/2N$ is, therefore, the frequency of *A* allele in the population, and is represented by *p*. Similarly, the ratio $(2R+H)/2N$ is the frequency of *a* allele, and is written as *q*. Therefore,

$$p=(2D+H)/2N \text{ or}$$
$$=(D+\tfrac{1}{2}H)/N \text{ and}$$
$$q=(2R+H)/2N \text{ or}$$
$$=(R+\tfrac{1}{2}H)/N$$

Therefore, $p+q=1$ or

$$p=1-q, \text{ or } q=1-p$$

The values of *p* and *q* are known as gene frequencies. *Gene frequency* is the proportion of an allele, *A* or *a*, in the population. In other words, the proportion of gametes carrying an allele, *A* or *a*, is known as gene frequency. The *genotype frequency* or *zygotic frequency* is the proportion of a genotype, *AA*, *Aa* or *aa*, in the population. Random mating or random union of the two types of gametes would produce the following genotypes in a ratio proportionate to the frequencies of gamates uniting to produce the zygote.

♂ / ♀	*p A*	*q a*
p A	p^2 *AA*	*pq Aa*
q a	*pq Aa*	q^2 *aa*

Thus the genotypic frequencies are : p^2 *AA*, $2pq$ *Aa* and q^2 *aa*.

According to the Hardy-Weinberg law, the frequencies of two types of gametes produced by this population would be *p* (for *A*) and *q* (for *a*). Therefore, the frequencies of genotypes in the next generation will be the same as those in the present generation,

that is p^2 *AA*, $2pq$ *Aa* and q^2 *aa*. This can be easily shown as follows. In the present generation,

Genotype	*AA*	*Aa*	*aa*
Genotypic frequency	p^2	$2pq$	q^2

This population would produce two types of gamets *A* and *a*. The frequencies of gametes containing *A* and *a* alleles can be calculated in a similar manner as described before. It may be noted that $D=p^2$, $H=2pq$ and $R=q^2$. Further, $N=1$ since,

$$p^2+2pq+q^2=(p+q)^2 \text{ and}$$
$$p+q=1, \text{ hence}$$
$$p^2+2pq+q^2=1$$

The frequencies of *A* and *a* gametes may be calculated as follows.

Frequency of gametes containing *A* allele

$$=(D+\tfrac{1}{2}H)/N$$
$$=(p^2+pq)/1$$
$$=p^2+pq$$

Similarly, the frequency of gametes containing *a* allele

$$=(R+\tfrac{1}{2}H)/N$$
$$=(q^2+pq)/1$$
$$=q^2+pq$$

It can be easily shown that $p^2+pq=p$.

$$p^2+pq=p\,(p+q)$$
$$=p \text{ (since } p+q=1)$$

Similarly, $q^2+pq=q\,(q+p)$

$$=q \text{ (since } q+p=1)$$

It is clear that the frequency of the two types of gametes is the same as in the previous generation. It can be similarly shown that the frequencies of the three genotypes in this generation would also be the same as those in the previous generation. Random union of the male and female gametes would produce the following genotypes.

♂ / ♀	*pA*	*qa*
pA	p^2 *AA*	*pq Aa*
qa	*pq Aa*	q^2 *aa*

Thus the genotypic frequencies would be p^2 *AA*, 2 *pq Aa* and q^2 *aa*, which is the same as in the previous generation. It can be shown that random mating between various genotypes would lead to the same result. The frequency of mating between identical genotypes, *e.g.*, between *AA* and *AA*, *Aa* and *Aa* or *aa* and *aa* would be equal to the square of the frequency of that genotype. For example, the frequency of mating $Aa \times Aa$ would be $(2pq)^2$, *i.e.*, $4p^2q^2$. But the frequencies of matings between two different genotypes, *e.g.*, *AA* and

Aa, *AA* and *aa* etc., would be twice the product of the frequencies of the two genotypes. This is because in such cases two combinations are possible. For example, the mating between *AA* and *aa* could be either as *AA* as a male ($aa \times AA$) or *aa* as male ($AA \times aa$). The frequency of this mating, therefore, would be 2 ($p^2 \times q^2$) or $2p^2q^2$. Table 8.1 lists the frequencies of various types of matings and the progeny produced from them.

Table 8.1. Consequences of random mating of genotypes in a Mendelian population,

Mating	*Frequency of mating*	*Frequency of progeny from the mating*		
		AA	*Aa*	*aa*
$AA \times AA$	$p^2 \times p^2 = p^4$	p^4		
$AA \times Aa$	$2(p^2 \times 2pq) = 4p^3q$	$2p^3q$	$2p^3q$	
$AA \times aa$	$2(p^2 \times q^2) = 2p^2q^2$		$2p^2q^2$	
$Aa \times Aa$	$(2pq \times 2pq) = 4p^2q^2$	p^2q^2	$2p^2q^2$	p^2q^2
$Aa \times aa$	$2(2pq \times q^2) = 4pq^3$		$2pq^3$	$2pq^3$
$aa \times aa$	$q^2 \times q^2 = q^4$			q^4

The frequency of progeny from a mating is deduced by a simple logic. For example, mating $AA \times AA$ would produce only one type of progeny, *AA*. The frequency of progeny from a mating would be proportionate the frequency of the mating, *e.g.*, p^4, in the above case. The matting $AA \times Aa$ would produce two types of progency *AA* and *Aa* in equal proportions. The frequency of the mating is $4p^3q$. Therefore, the frequency of progeny would be $2p^3q$ *AA* and $2p^3q$ *Aa*. Similarly, the frequencies of different progenies produced by other matings could be worked out.

The frequency of progeny with *AA* genotype would be,

$$= p^4 + 2p^3q + p^2q^2$$
$$= p^2(p^2 + 2pq + q^2)$$
$$= p^2 \quad (\text{since } p^2 + 2pq + q^2 = 1)$$

Similarly, the frequency of *aa* progeny would be,

$$= p^2q^2 + 2pq^3 + q^4$$
$$= q^2(p^2 + 2pq + q^2)$$
$$= q^2 \quad (\text{since } p^2 + 2pq + q^2 = 1)$$

And the frequency of *Aa* progeny would be,

$$= 2p^3q + 2p^2q^2 + 2p^2q^2 + 2pq^3$$
$$= 2p^3q + 4p^2q^2 + 2pq^3$$
$$= 2pq\ (p^2 + 2pq + q^2)$$
$$= 2pq \ (\text{since } p^2 + 2pq + q^2 = 1)$$

Thus it can be seen that random mating of genotypes also produces the three genotypes *AA*, *Aa* and *aa* in the frequencies p^2, $2pq$ and q^2,

respectively. Thus it is clear that in random mating populations the gene and genotype frequencies remain constant generation after generation.

When genotype frequencies are disturbed and the population is not at equilibrium, it reaches equilibrium after one generation of random mating. But when we consider two genes together, the equilibrium is not restored in one generation, but the approach to equilibrium is very rapid. Linkage reduces the rate by which a population reaches equilibrium. However, when many genes are considered together, the approach to equilibrium is very slow. It may be pointed out that strict random mating seldom, if ever, occurs in cross-pollinated crop species, particularly in the small populations that breeders maintain. Furthermore, the genotype and gene frequencies are affected by selection, mutation and migration. We shall briefly consider the effects of these forces on gene and genotype frequencies in Mendelian populations.

FACTORS DISTURBING THE EQUILIBRIUM IN POPULATIONS

The equilibrium in radom mating populations is distubred by migration, mutation, selection and random drift ; these factors are also referred to as *evolutionary forces.*

Migration

Migration is the movement of individuals into a population from a different population. Migration may introduce new alleles into the population or may change the frequencies of existing alleles. The amount of change in gene frequency q will primarily depend upon two factors : *first*, the ratio of migrant individuals to those of the original population and *second*, the magnitude of difference between the values of q in the population and in the migrants. In plant breeding programmes, migration is represented by intervarietal crosses, polycrosses etc., wherein the breeder brings together into a single population two or more separate populations.

Mutation

Mutation is a sudden and heritable change in an organism and is generally due to a structural change in a gene. It is the ultimate source of all the variation present in the biological materials. *Mutation may produce a new allele not present in the population or may change the frequencies of existing alleles.* However, since the mutation rate is generally very low, *i.e.*, approximately 10^{-6}, the effects of mutation on gene frequency would be detectable only after a large number of generations. Therefore, in breeding populations such effects may be ignored. A desirable mutation may prove very useful when it is discovered. But a systematic use of mutuations in crop improvement would not be feasible until techniques for directed mutagenesis have been perfected. Directed mutagenesis implies that the experimenter should be able to induce a high frequency of the desired mutations through certain techniques. At present, directed mutagenesis is an ideal which is yet to be achieved even partially.

Random Drift

Random drift or genetic drift is a random charge in gene frequency due to sampling error. Random drift occurs in small populations because sampling error is greater in a smaller population than in a large one. Ultimately, the frequency of one of the alleles becomes zero and that of the other allele becomes one. The allele with the frequency of one is said to be *fixed* in the population because there would be no further change in its frequency. It may be expected that in a small population all the genes would become homozygous, or would be fixed in due course of time. Breeding populations are generally small, hence a certain amount of genetic drift always occurs in them. The breeder cannot do anything to prevent this genetic drift, except to use very large populations which is often not practicable. Alternatively, he may resort to phenotypic disassortative mating which would again require time, labour and money.

Inbreeding

Mating between individuals sharing a common parent in their ancestry is known as inbreeding. In small populations, a certain amount of inbreeding is bound to occur. Inbreeding reduces the proportion of heterozygotes or heterozygosity and increases the frequency of homozygotes or homozygosity. The rate of decrease in heterozygosity is equal to $1/2N$ (N=number of plants) per generation in monoecious or hermaphrodite species. In dioecious species and in monoecious species where self-pollination is prevented, the decrease in heterozygosity is somewhat lower ; it is equal to $1/(2N+1)$ per generation. Thus in small populations, even with strict random mating or even with outcrossing the frequency of homozygotes increases, while that of heterozygotes decreases due to inbreeding.

Selection

Differential reproduction rates of various genotypes is known as selection. In crop improvement, selection is very important because it allows the selected genotypes to reproduce, while the undesirable genotypes are eliminated. Thus the breeder is able to improve the various characteristics by selecting for the desirable types. In a random mating population, if plants with *AA* or *aa* genotypes are selected, the frequency of *A* allele in the selected population would be 1 or 0, respectively. It is assumed in this case that *AA* and *aa* genotypes would be identified without error. In the next generation, therefore, only *A* or *a* allele would be present, *i.e.*, the alleles would be fixed. Here the selection against the remaining genotypes is complete, that is, these genotypes are not allowed to reproduce. In such cases, the disadvantage in reproduction, *i.e.*, selection differential *s*, is 1 and the fitness is zero for the remaining genotypes. *The fitness of a genotype may be defined as its reproduction rate in relation to that of other genotypes.* Generally, *s* has values less than

one. Further, often it is not possible to identify the genotypes with certainty. The identification of genotypes is made difficult by dominance and due to less than 100 per cent heritability. This is particularly true for quantitative characters. As a result, selection is expected to change gene frequencies rather than to eliminate one or the other allele.

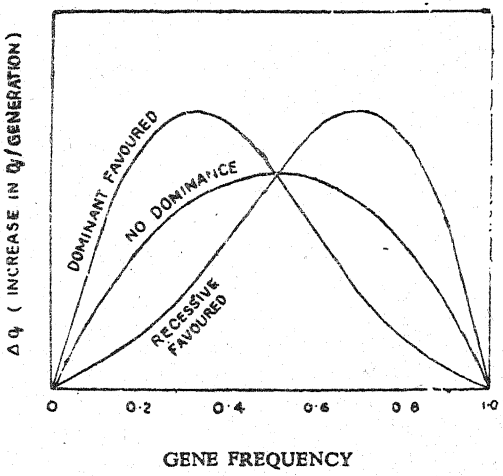


Fig. 8.1. Change in q ($\triangle q$) per generation under constant selection intensity (s) as affected by dominance.

When s is less than 1, the rate of change in gene frequency, *i.e.*, $\triangle q$, would depend upon the intensity of selection (s) and upon the gene frequency q. If s is fixed, that is, for a given value of s, $\triangle q$ would depend upon the gene frequency q and would be affected to some extent by the degree of dominance. The effects of gene frequency and dominance on $\triangle q$ are shown in Fig. 8.1. It can be seen that when an allele is rare in a population, $\triangle q$ due to selection for this allele is small. As the value of q increases due to selection, the $\triangle q$ also increases reaching a maximum at about $q=0.3$ in the case of dominant allele A, at $q=0.5$ when there is no dominance and at $q=0.7$ in the case of recesive allele a. After this, $\triangle q$ again declines as the favoured allele becomes more frequent in the population. It is evident that the effect of dominance is to determine the value of q at which $\triangle q$ would be the maximum. *Thus selection in a random mating population is highly effective in increasing or decreasing the frequency of alleles, but it is unable to either fix or eliminate them. However, in combination with a system of inbreeding, selection is highly efficient in the fixation and elimination of alleles.*

In case selection favours the heterozygote *Aa*, both the alleles are retained in the population. The q and p reach epuilibrium when $q=sa/(sa+sA)$, where sa and sA are the selection

intensities against *oa* and *AA*, respectively. Thus the equilibrium value of q is often different from 0.5 although at $q=0.5$ the frequency of heterozygote *Aa*, the most favoured class, is the maximum. This is because the fitness of the population as a whole is the greatest at the equilibrium frequency of q, although the frequency of heterozygote may not be the maximum.

Most of the characters of economic importance are quantitative characters and are governed by many genes. Such characters show a continuous distribution. Therefore, selection intensity is measured as the difference between mean of the population and that of the selected individuals. This is more conveniently expressed in terms of standard deviation units from which the selection differential k is calculated. The value of k is related to the per cent of extreme individuals saved, *e.g.*, when the extreme 5% of the population is saved, $k=2.06$.

Selection of the extreme phenotype increases the frequency of desirable alleles in the population. The $\triangle q$ for each gene would become smaller as the number of genes governing the character increases. With an increase in the frequency of desirable alleles, the frequency of desirable genotypes would also increase. New genotypes would also appear producing more extreme phenotypes. Therefore, mean of the population would change under selection. $\triangle q$ would be the greatest when $q=0.5$ since the gene action is assumed to be additive. With $q<0.5$ or >0.5, the $\triangle q$ for each gene would become smaller. Thus selection will be unable to fix or eliminate alleles in the case of characters governed by several genes. Furthermore, variance may decrease to some extent, but selection would show continuous gain for several generations. In case the quantitative character is governed by one or few genes with large effects, $\triangle q$ would be relatively large and the desirable alleles may be fixed after some generations of selection. The gain under selection in such a case would continue for several generations, but in the later generations it would be very small and, more likely, discouraging.

The progress under selection for quantitative characters is retarded by (1) nonadditive gene action, (2) low heritability, and (3) the limit on permissible selection intensity. *Heritability* of quantitative characters is always less than 100 per cent. Therefore, there is less than perfect correspondence between genotype and phenotype. This makes the identification of desirable genotypes difficult and, therefore, reduces the progress under selection. *Permissible selection intensity* is dependent upon fecundity, *i.e.*, reproductive capacity, of the species. Suppose the breeder desires to grow 1,000 individuals in every generation. The number of plants selected must be large enough to produce 1,000 offspring. That is, if 5% of the population is saved, there would be 50 plants (5% of 1,000) to produce the next generation. Each plant must produce at least 20, *i.e.*, 1,000/50, seeds to obtain 1,000 progeny. In most of the crop plants, this is not a problem, but in many of the animal species such a high reproduction

rate is impossible. Another factor that limits the selection intensity is the consideration of inbreeding. *Inbreeding* should be kept to a low level to maintain heterozygosity and to avoid inbreeding depression. As a result, the number of selected plants should be relatively large and cannot be too small.

The above considerations are for a single character. When two characters have to be selected for simultaneously, the selection intensity for each of the two characters has to be relaxed. The breeder generally has to concentrate on the improvement of more than one character. Therefore, the intensity of selection for any one character is much lower than that for a single character. For example, if selection is practised for three characters, and 5 per cent of the population is saved in each generation, the intensity of selection for any one character would be $3\sqrt{0{\cdot}05}$ or 37 per cent and not 5 per cent. This is because the selection for each character has to be relaxed in order to accommodate selection for the other two characters.

SYSTEMS OF MATING

The breeder has two basic tools to change the genetic composition of populations : (1) selection, and (2) mating system. We have discussed the effects of selection in some detail. Here we would examine the consequences of various systems of mating. There are five basic mating schemes : (1) random mating, (2) genetic assortative mating, (3) genetic disassortative mating, (4) phenotypic assortative mating, and (5) phenotypic disassortative mating.

Random Mating

In random mating, each female gemete is equally likely to combine with any male gemete and the rate of reprodution of each genotype is equal, i.e., there is no selection. In such a situation, (1) gene frequencies remain constant, (2) variance for the character is constant, and (3) the correlation between relatives or prepotency does not change. However, in plant breeding some form of selection is practised ; such a mating system is known as *random mating with selection.* Further, the mating is usually not completely random. Differenes in flowering time, position of plants in the field, self-incompatibility, and prevalent wind direction etc. limit the randomness of mating.

Random mating with selection changes the mean of the character, and increases the frequency of alleles for which selection is practised. It would tend to increase the variance when the selection is in favour of a rare allele, but would tend to reduce it when a predominant allele is selected for. These changes are more pronounced when the character is highly heritable and is governed by one or a few genes. In case of polygenic traits with

moderate heritability, the change due to selection is relatively smaller. Random mating in small populations is unable to prevent an increase in homozygosity due to inbreeding and genetic drift. Genetic drift may often lead to a chance fixation on alleles.

Random mating is useful in plant breeding in several ways, e.g., progeny testing, production and maintenance of synthetic and composite varieties, production of polycross progeny etc.

Genetic Assortative Mating

In genetic assortative mating, the mating is between individuals that are more closely related by ancestry than in random mating. Thus in this mating system, it is not important that the genotype of the plants selected for mating be correctly identified. This mating system is more commonly known as *inbreeding*. Inbreeding has the following effects on a population.

1. It increases homozygosity and reduces heterozygosity.
2. Alleles and, as a result, the characters become fixed, except for the variation produced by environment. The fixation of a character is little affected by the number of genes involved and the heritability of the character, particularly under more intense forms of inbreeding, *e.g.*, self-fertilization.
3. Under intense inbreeding, the number of noninterbreeding groups increases rapidly. As a result, selection must be done to keep the population size manageable.
4. The total genetic variability of the population increases rapidly. The genetic variability within interbreeding groups, *i.e.*, families or lines, decreases rapidly and is ultimately reduced to zero.
5. Under selection, genetic variability of the population decreases rapidly. In cases where only one line is selected, the genetic variability is very small or zero.
6. The prepotency of individuals increases under inbreeding. *Prepotency* is the property of an individual to produce progeny which are similar to each other and to the parent. Prepotency is affected by homozygosity, dominance, epistasis and linkage. Homozygosity is the most important factor and is under control of the breeder. As homozygosity increase the prepotency of an individual also increases. An individual completely homozygous for all the dominant alleles will be the most prepotent.

Genetic assortative mating is useful in the development of inbreds, both partial and complete.

Genetic Disassortative Mating

In genetic disassortative mating, such individuals are mated which are less closely related by ancestry than would be under random mating. Thus in this system, totally unrelated individuals are mated. These individuals often belong to different populations. Examples of

such a mating are intervarietal and interspecific crosses. The effects of genetic disassortative mating are similar to those of migration. *It may be expected that this mating system would reduce homozygosity and increase heterozygosity.*

Phenotypic Assortative Mating

Mating betweeen individuals which are phenotypically more similar than would be expected under random mating is called phenotypic assortative mating. Phenotypic assortative mating leads to,

1. Division of the population into two extreme phenotypes. Unlike inbreeding which fixes intermediate types as well, under this system of mating the intermediate types are not fixed. Only the two extreme phenotypes, *i.e.*, the lowest and the highest, remain in the population.
2. Increase in homozygosity.
3. Increase in genetic variability provided both the extreme phenotypes are kept in the population.
4. An increase in the prepotency of individuals due to the increase in homozygosity.

These effects disappear rapidly when random mating is restored. The effects of phenotypic assortative mating become quickly obvious in the case of those characters that are governed by one gene, but the effects are slower to appear in the case of those governed by two genes. In the case of polygenic traits, the effects are much slower to realise, and the intermediate types are seldom eliminated. Dominance and nonadditive gene actions further reduce the effects of phenotypic assortative mating. Since heritability is always less than 100 per cent for most of the quantitative traits, the effectiveness of this mating is further reduced. Therefore, the increase in homozygosity etc. is much slower than that expected with 100 per cent heritability.

This mating system is useful in the isolation of extreme phenotypes. It is used in some breeding schemes, e.g., in recurrent selection.

Phenotypic Disassortative Mating

Mating between phenotypically dissimilar individuals is referred to as phenotypic disassortative mating. The consequences of this mating are as follows.

1. Maintenance of or even some increase in heterozygosity.
2. Some reduction in population variance since it tends to produce intermediate phenotypes.
3. Reduction in correlation between relatives or in prepotency due to the increase in heterozygosity.

This mating system is very useful in making a population stable, i.e., in maintaining variability. Suitable parents may be selected to remove their weaknesses. The progeny from such a mating would be more desirable than the parents. It is also useful when the desirable

type is an intermediate one and the available parents have the extreme phenotypes. But the most notable use of this mating system is in maintaining variability in relatively small populations as it reduces inbreeding.

SUMMARY

In random mating populations, gene and genotype frequencies remain constant if there is no migration, mutation, genetic drift and selection. *Mutation* is of little significance in plant breeding programmes. *Migration* is represented by intervarietal and interspecific crosses. It may introduce new alleles in the population and may change gene and genotype frequencies. *Genetic drift* and *inbreeding* are associated with small populations. They cause an increase in homozygosity.

Selection is the most important tool in plant breeding. Selection is effective in changing gene and genotype frequencies. As a result, it alters the mean in the direction of selection. New genotypes appear due to new gene combinations. The variance may also be affected. If a character is governed by one or a few major genes, selection would lead to the fixation of desirable alleles. But in case of polygenic traits, fixation does not occur. Advance under selection is limited by dominance, nonadditive gene action and less than 100 per cent heritability. Of these three factors, heritability is of the greatest importance.

Genetic assortative mating increases homozygosity and is useful in the development of inbred lines. *Phenotypic assortative mating* eliminates the intermediate types and divides the population into two extremes. This mating is desirable when the extreme types are desirable. *Genetic disassortative mating* is represented by intervarietal crosses. *Phenotypic disassortative mating* maintains heterozygosity in the population. It is a suitable mating scheme in cases where the population is to be kept stable and heterozygous.

QUESTIONS

1. Explain the Hardy Weinberg law in detail with the help of suitable formulae.
2. List the factors which disturb the Hardy-Weinberg equilibrium in a Mendelian population and briefly describe their effects.
3. What are the different systems of mating ? Describe the genetic consequences of these mating systems.
4. What are the differences between the following : (i) migration and introgression, (ii) gene pool and gene frequency, (iii) prepotency and fecundity, and (iv) selection differential and selection intensity.
5. The equilibrium in a random mating population is disturbed by selection, migration, mutation, genetic drift and inbreeding. In which of the above cases, it will be possible to return the population to the original composition and how ? Explain with reasons.
6. Describe the following in relation to population genetics : selection differential, selection intensity, random drift, migration, permissible selection intensity, fecundity, prepontency, fitness of a genotype, gene frequency, zygotic frequency.
7. In a random mating population, a character is governed by a single gene with two alleles *A* and *a*. Assuming that the frequency of *A* is *p* and that of *a* is *q*, compute the genotypic frequencies in the population. Show that these frequencies will remain unchanged in the next generation assuming random union of gametes as well as random mating among genotypes.

Suggested Further Reading

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CHAPTER 9

Selection in Cross-Pollinated Crops

Selection in a random mating population is able to (1) change the gene and genotype frequencies, (2) produce new genotypes due to the changed gene frequencies, (3) cause a shift in mean in the direction of selection, and (4) change the variance of population to some extent (Chapter 8). The magnitude of these effects is influenced by the number of genes controlling the character, the degree of dominance; the nature of gene action and, to a large extent, heritability. The effects of these factors may be summarised as follows.

Characters Governed by One or Few Genes. The gain under selection would be slow if the allele being selected for is rare. The gain would become more rapid as the frequency of selected allele increases. It will again become slow as the selected allele becomes predominant in the population. After some, *e.g.*, 4-6, generations under selection, the gain would be small; the genetic variance would also be much smaller than that in the original population.

Polygenic Traits. When the character is governed by many genes, the gain under selection would be small and would continue for many generations. The mean would change in the direction of selection. But the variance of the population would not be affected to any appreciable extent.

Dominance and nonadditive gene actions would tend to slow down the progress under selection.

Heritability. Heritability is of great importance in determining the progress under selection. Less than 100 per cent heritability will cause a reduction in the progress under selection. If the heritability is low, there may be little or no gain under selection.

A large number of studies on the effect of selection in random mating populations have been published. The responses to selection obtained in these studies can be divided into five broad groups : (1) rapid gain, followed by slow progress, (2) continued slow response for a long period, (3) slow response for a short period,

(4) little or no respone, and (5) rapid gain—plateau—rapid gain response.

RAPID GAIN FOLLOWED BY SLOW RESPONSE

In some cases, selection produces rapid gains for some generations. This is followed by a period of slow gain under selection (Fig. 9.1). This type of response is typical of characters like plant

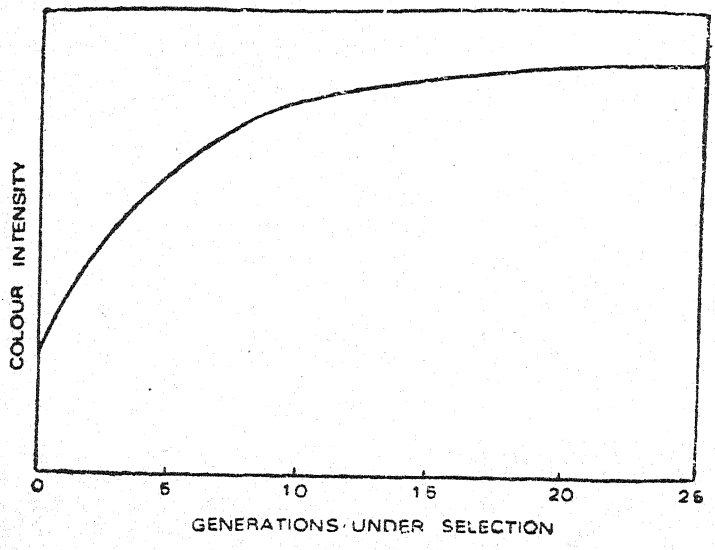


Fig. 9.1. A diagrammatic representation of rapid initial gain followed by slow response for several generations.

height, resistance to certain diseases, days to flowering, colour etc. Selection in the case of these characters leads to the following.

1. Change in the mean in the direction of selection.
2. Appearance of new genotypes or gene combinations due to the changed gene frequencies.
3. Reduction in variability as indicated by variance.

Genetic Interpretation. It is suggested that such characters are governed by few genes with major effects and several genes wi th small effects. The period of rapid gain represents an increase in the frequency of desirable alleles of the genes with large effects. This increase would be large at intermediate gene frequencies; it would become progressively smaller as the desirable alleles become predominant in the population. The slow gain following the rapid gain is due to two factors. *First*, there is a slow increase in the frequency of major genes since they have become predominant.

Second, there is an increase in the frequency of minor genes, but the rate of change in the frequency of each minor gene is bound to be small. The slow gain, therefore, continues for a long period because the genes would reach fixation only after a long period under selection.

CONTINUED SLOW PROGRESS FOR A LONG PERIOD

A suitable example of this kind of response is presented by selection for high oil and high and low protein contents in Burr White, an open-pollinated variety of maize (*Z. Mays*). We shall consider here the findings on selection for oil content. The original population of Burr White had a mean oil content of 4.7 per cent and a range of 3.7 to 6.0 per cent. The mean oil content of the population increased steadily at a low rate for 76 generations under selection (Fig. 9.?) reaching a value of 19.0 per cent. Plants with more than 6.0 per cent oil content were observed in the second generation under selection. By the tenth generation under selection, all the plants in the population had higher than 6 per cent oil content. This indicates the *appearance of new genotypes* not present in the original population. *The variability* in the selected population after 50 generations of selection was comparable to that in the original population. The evidence for this was obtained in three ways ; *first*, the variances for the different generations were comparable ; *second*, suspension of selection resulted in some decrease in oil content, *i.e.*, a tendency to revert to the original Burr White ; and *third*, selection for low oil content was effective. These findings clearly indicate that a considerable variability for oil content was present even after 50 generations of selection. Similar results were obtained with high and low protein contents ; there was considerable genetic variability even after 76 generations of selection. The effects of selection may be summarised as follows.

1. Slow change in mean in the direction of selection.
2. Appearance of new gene combinations after some generations under selection.
3. Maintenance of variability even after long periods under selection.

Genetic Interpretation. Quantitative characters are generally governed by several genes ; each gene has small additive effect. In such a case, $\triangle q$ for each gene would be small. Consequently, progress under selection for such traits would be slow. The progress would be further slowed down due to less than 100 per cent heritability. As a result, genes would be seldom fixed, and there would be considerable variability even after several generations under selection. It has been observed that selection for one quantitative character generally leads to changes in other unrelated characters. This is referred to as *correlated response to selection.* For example, selection

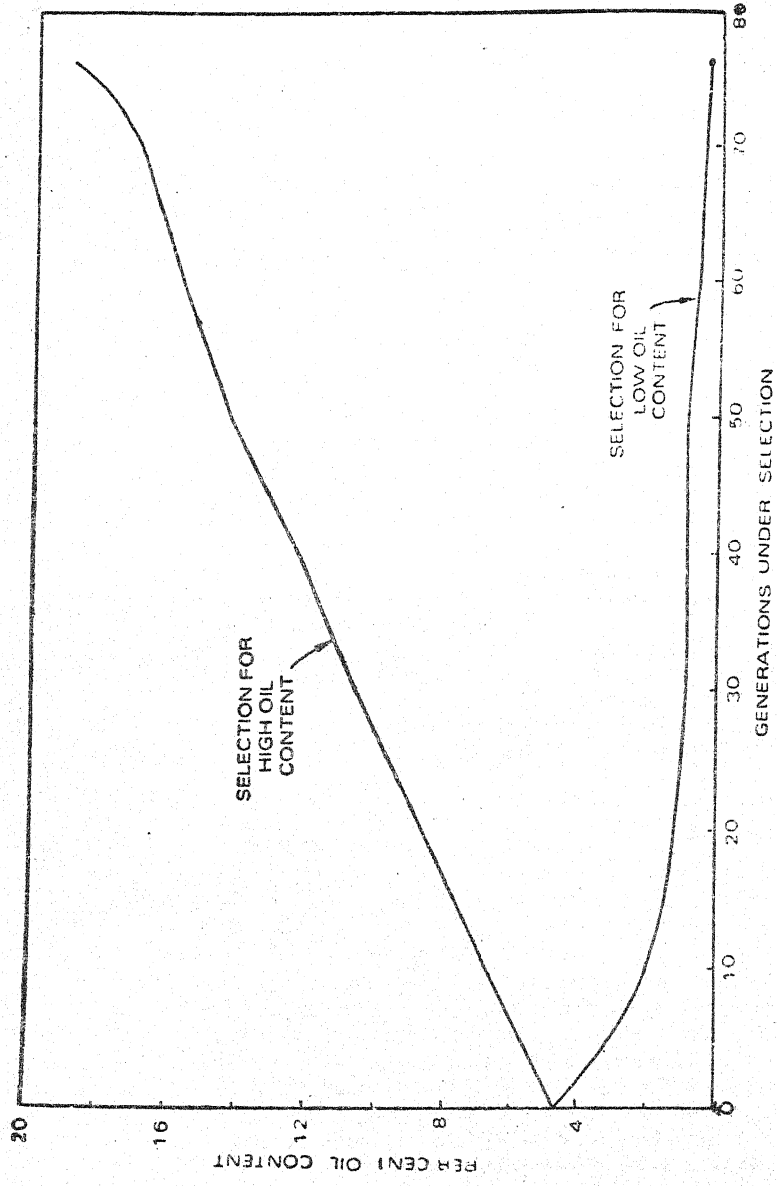

PER CENT OIL CONTENT
20
16
12
8
4
0
10
20
30
40
50
60
70
80
GENERATIONS UNDER SELECTION
SELECTION FOR HIGH OIL CONTENT
SELECTION FOR LOW OIL CONTENT

Fig. 9.2.

for high and low oil and high and low protein contents in Burr White maize brought about changes in such characters as maturity, plant height, tiller number, cob size, grain characters and yield.

SLOW RESPONSE FOR A SHORT PERIOD

Selection for some characters shows slow gain for several generations which ends in a plateau. Further selection for the character is not effective. Selection for low oil content in Burr White maize is an example of such a response. Oil content decreased slowly upto 25 generations of selection ; it had decreased to about 1.0 per cent in the selected population from 4.68 per cent in the original Burr White. Continued selection for another 25 generations was ineffective in further reducing the oil content below one per cent (Fig. 9.2). The effects of selection on the population are similar to those in the previous case, that is, slow response for a long period under selection.

Most of the oil in maize seed is found in the embryo. Selection for low oil content was accompanied with a reduction in embryo size. Seed viability is likely to decrease with a decrease in embryo size. There would be a minimum threshold embryo size below which it will not survive. As a result, the embryo size is likely to restrict the reduction in oil content by reducing the viability of embroys with low oil content.

Genetic Interpretation. The oil content is likely to be controlled by several genes (possibly more than 50 genes). Selection is expected to produce slow gains for several generations. The variability will not be affected substantially by selection. That this is so was evident from a comparison of the variances from selected and unselected populations, trend of reversion upon suspension of selection, and the success of selection in the reverse direction. Thus there was a considerable variability for low oil content even after 25 generations under selection. But the progress under selection was checked due to physiological limitations, *i.e.*, reduced embryo survival due to reduced embryo size as a consequence of the reduction in oil content.

Fig. 9.2. Effects of selection for high and low oil contents in the open-pollinated maize variety Burr White. (Approximated from J.W. Dudley, 1977. Proc. Int. Conf. Quantitative Genetics, eds. E. Pollack, O.Kmpthorne and T B. Bailey, Jr., The Iowa State Univ. Press. Ames, pp 459-473) The selection for high oil content represents slow gain for many generations, while that for low oil content represents slow response for a period followed by a plateau. Continued selection for low oil produced a very slow response after 60 generations so that after 76 generations the mean for low oil line was 0.3 per cent (as compared to about 1 per cent after 50 generations). The estimated minimum number of genes differing between high and low oil populations is 54, while that between high and low protein lines is 122 ! The genetic gain udder selection was $20 \times \sigma P$ for high oil and high protein! (σP is the phenotypic standard deviation)

LACK OF RESPONSE TO SELECTION

Selection for some characters produces little or no gain. A typical example of this type of response is provided by selection for yield in maize. A large number of experiments on selection for yield have been done. In general, selection failed to bring about an increase in yield. For example in one experiment, the yield of selected population after seven generations of selection was 53.3 bushels (one bushel=36.35 litres) per acre as compared to 53.6 bushels for the unselected variety.

Genetic Interpretation. Several experiments have shown that open-pollinated varieties of maize possess considerable genetic variance for yield. A major protion of this variance is additive, that is, would show response to selection. Thus the failure of yield to respond to selection is most likely due to the low heritability of this trait. Low heritability makes it very difficult to identify the superior yielding genotypes. Therefore, the superiority of plants selected on the basis of phenotype would often be due to factors other than their genotype. This would limit the progress under selection. Breeding schemes utilizing replicated yield trials, *e.g.*, recurrent selection, are capable of improving yield by selection. This shows that the lack of response to selection for yield and some other characters is primarily due to their low heritability, which makes it very difficult to identify superior genotypes on the basis of phenotypes of individual plants.

RAPID—GAIN—PLATEAU—RAPID GAIN RESPONSE

Selection for some characters shows a period of rapid gain, followed by a plateau, which is followed by another period of rapid gain. Selection for increased abdominal bristle number in *Drosophila melanogaster* (fruit fly) by Mather and Harrison presents a typical example of this kind of response. The bristle number increased rapidly for 20 generations. But reproduction rate, *i.e.*, fertility, decreased sharply so that selection had to be stopped to maintain the selected population. After a few generations, fertility was restored, but the bristle number was now much lower than that in the selected population before selection was stopped. Selection for bristle number in this new population was highly effective, but this time there was no decrease in fertility. In a few generations of selection, the bristle number increased to a new stable level. Further selection for bristle number was ineffective, and the bristle number did not decrease when selection was stopped. After 50 generations at this plateau, the bristle number again showed a rapid response to selection and reached yet another plateau (Fig. 9.3). The main features of this experiment were as follows.

1. Rapid increase in bristle number under selection.

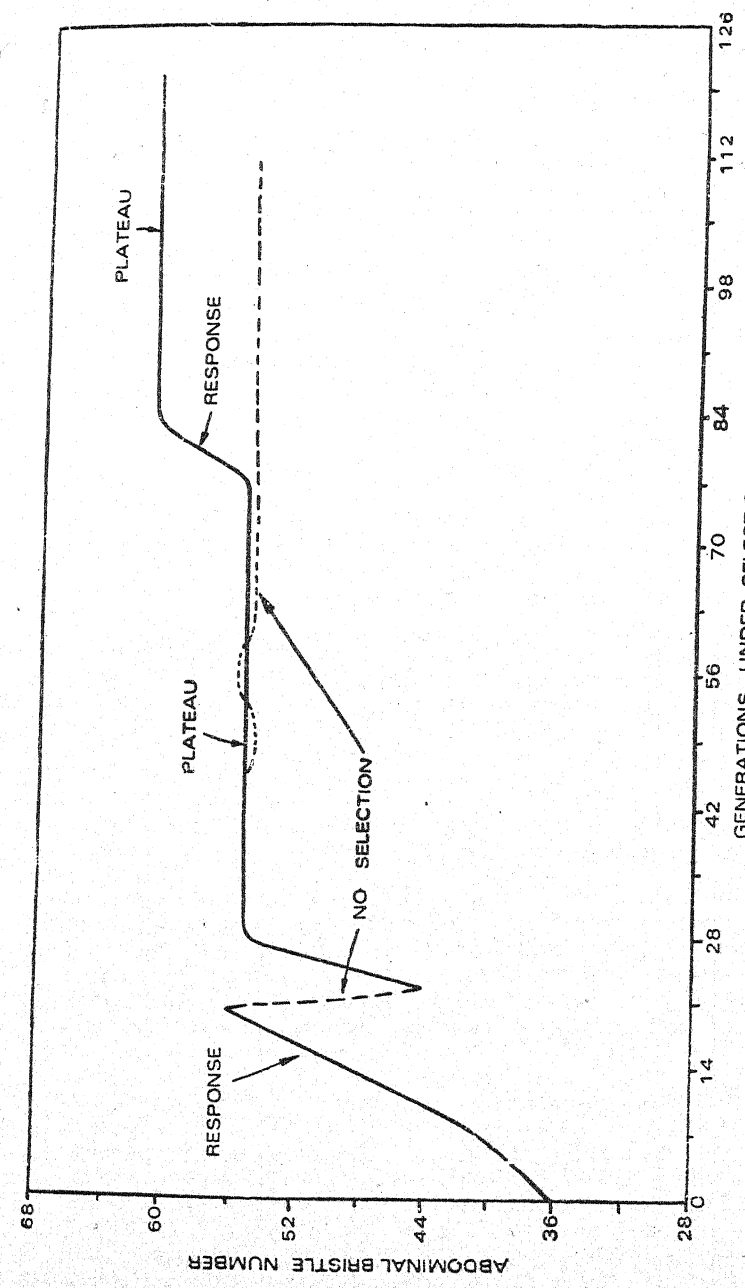


Fig. 9.3. Response to selection for abdominal bristle number in *Drosophila melanogaster*. The solid line represents the population under selection, while the broken line denotes that selection has been suspended (Approximated from K. Mather and B.I. Harrison, 1949, Heredity, 3 : 1-52, 131-162).

2. Decrease in fertility associated with the increase in bristle number.
3. Increase in fertility when selection was suspended, and the accompanied decrease in bristle number.
4. Rapid gain under selection without any decrease in fertility when selection was resumed in the new population.
5. Reaching a stable bristle number at which stage further selection was ineffective.
6. After several generations, another rapid gain under selection ending in yet another plateau.

Genetic Interpretation. It is believed that in natural populations, the best adapted or the most fit individuals are those that are close to the population mean for various quantitative characters. In other words, natural selection acts against individuals with the extreme phenotypes. Therefore, a population has to meet two opposite needs. *First*, for immediate adaptation it should become phenotypically uniform, *i.e.*, the individuals should be close to the population mean for the quantitative characters. And *second*, to meet the long term evolutionary requirements, the population must maintain genetic variability. It has been suggested that the population may be able to meet these opposite requirements if only a small part of the genetic variability is free, *i.e.*, is expressed, and the major portion is hidden as potential variability. *Free variability* is that portion of variability which is expressed as phenotypic difference between homozygotes with extreme phenotypes. The free variability is, therefore, exposed to selection. The *potential variability* is hidden in heterozygotes or in homozygotes which do not have the extreme phenotypes and, therefore, is not exposed to selection.

Types of Potential Variability. The Potential variability is of two types. : heterozygotic and homozygotic.

Heterozygotic Potential Variability. This variability is stored in heterozygotes, *e.g.*, *Aa Bb* etc. The heterozygotes *Aa* or *Aa Bb* etc. are phenotypically uniform and are very close to the population mean. But they would produce extreme phenotypes in the next generation due to segregation and recombination. Thus the heterozygotes function as stores of variability which is released slowly as free variability due to segregation and recombination.

Homozygotic Potential Variability. In case of two or more genes, homozygotes also function as stores of variability. For example, homozygotes *AA bb* and *aa BB* may be expected to cluster around the mean of the population. They would, therefore, be protected from natural selection, and would be phenotypically uniform. However, they would produce the extreme phenotypes *AA BB* and *aabb* after hybridization, *i.e.*, *AA bb*$\times$*aaBB*, followed by segregation and recombination in the F_1. The release of homozygotic variability.

therefore, requires it to be first converted into heterozygotic variability through hybridization. As a result, it is converted into free variability more slowly than heterozygotic variability.

In the case of polygenic characters, several genes governing a character may be present on the same chromosome. It will be advantageous to the population if these genes were linked in the repulsion phase, *i.e.*, some dominant genes were linked with some recessive genes. For example, out of the four schemes for the arrangement of four genes, *A*, *B*, *C* and *D*, given below, scheme number 4 would be the most desirable. This is because in this scheme, the full release of variability would require three crossovers at precise points (marked×). It may be expected that natural populations would develop such complex and elaborate gene arrangements for storing variability. This would permit them to meet the opposing demands of immediate fitness and long-term evolutionary requirements.

(1) $\frac{ABCD}{abcd}$ (2) $\frac{ABcd}{abCD}$ (3) $\frac{aBCd}{AbcD}$ (4) $\frac{A}{a} \times \frac{b}{B} \times \frac{C}{c} \times \frac{d}{D}$

It is suggested that plus and minus genes affecting a quantitative character are linked in a manner similar to that in the scheme 4 above. The genes for other quantitative characters would also be linked with these genes, and would perhaps be distributed in between these genes. The initial rapid gain under selection in the case of bristle number in *Drosophila* was most likely due to the selection for blocks of genes linked together rather than for individual genes. The associated reduction in fertility represents the correlated response to selection, which is easily explained by the above gene organisation. Since the genes governing various quantitative characters would be linked together, selection for one character would also affect the other characters. The effects of such a correlated response to selection may be eliminated by crossing over among the linked genes. When selection was suspended, crossing over would have occurred to produce new gene combinations. As a result, selection for bristle number in the new population did not lower fertility. The second rapid gain represents further recombination among linked genes releasing some more of the potential variability.

SUMMARY

Selection in cross-pollinated species (1) changes the mean in the direction of selection, (2) increases the frequency of desirable alleles and genotypes, (3) leads to the production of new genotypes, and (4) may or may not reduce genetic variance. There are five different types of responses to selection. *First*, rapid gain followed by a period of slow gain is seen in characters governed by few major genes and several minor genes. The *second*, slow gain for a long time is characteristic of traits governed by polygenes; contribution of each gene is small but the heritability is high. The *third* type of response is slow gain ending in a plateau. This is found in case of those quantitative characters where change due to selection beyond a limit is prevented by physiological limitations. The *fourth* type of response is characteristic of those quantitative characters that have low heritability, *e.g.*, yield. In such characters, selection produces little or no gain. The failure of selection is primarily due to low

heritability. The *fifth* type of response, rapid gain—plateau—rapid gain, results from the release of potential variability. The initial response to selection is due to free variability present in the form of blocks of linked genes. The plateau presents the time during which the potential variability is slowly released by recombination or crossing over. This release of potential variability is followed by another rapid gain under selection. Selection for one character is likely to bring about change in other characters as well. This is known as *correlated response to selection*, which is due to linkage among genes governing different characters.

QUESTIONS

1. List the various types of responses to selection in cross-pollinated populations. Describe any two of them and give the genetic basis.
2. Describe the rapid gain—plateau—rapid gain response to selection. Discuss its genetic basis with special reference to the mechanism of storage of variability.
3. Write short notes on the following : (i) correlated response to selection, (ii) long continued response to selection, (iii) lack of response to selection, (iv) free variability, and (v) potential variability.

Suggested Further Reading

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CHAPTER 10

Heterosis and Inbreeding Depression

Cross-pollinated species and species reproducing asexually are highly heterozygous. These species usually show a severe reduction in fertility and vigour due to inbreeding (*inbreeding depression*). Conversely, hybridization between unrelated strains generally leads to an increased vigour and fertility (*hybrid vigour*). These two aspects are of great significance in breeding of these species. Most of the improved varieties in these crops are either hybrids or composites, both utilizing the phenomenon of hybrid vigour. Therefore, we would consider in some detail the features of inbreeding depression and hybrid vigour or heterosis and their genetic and biochemical bases.

INBREEDING DEPRESSION

Inbreeding is mating between individuals related by descent or ancestry. When the individuals are closely related, *e.g.*, in brother-sister mating or sib mating, the degree of inbreeding is high. The highest degree of inbreeding is achieved by selfing. The chief effect of inbreeding is an increase in homozygosity in the progeny, which is proportionate to the degree of inbreeding. In fact, the measure of degree of inbreeding is provided by the degree of homozygosity in the progeny. For example, selfing reduces heterozygosity by a factor of $\frac{1}{2}$ in each generation (Chapter 6). The degree of inbreeding increases in the same proportion. In fact, the degree of inbreeding in any generation is equal to the degree of homozygosity in that generation.

Inbreeding depression may be defined as the reduction or loss in vigour and fertility as a result of inbreeding.

Historical

Inbreeding depression has been recognised by man for a long time. It may not be surprising in view of the harmful effects

produced by inbreeding. In many societies, marriages between closely related individuals have been prohibited since early times. Hindu society perhaps presents the extreme example where marriages between individuals related by ancestry, howsoever distant, is prohibited. But systematic observations on the effects of inbreeding date back to about 1700 A.D. when inbreeding became a common practice in cattle breeding. There were considerable improvements in production, but there was a definite decline in fertility. Sooner or later it became necessary to outcross the inbred lines to increase fertility. In 1876, Darwin published his book *Cross and Self Fertilization in Vegetable Kingdom* ; he concluded that progeny obtained from self-fertilization were weaker than those derived from outcrossing. Darwin also reported the results from his experiments on self- and cross-fertilization in maize ; these are the first published accounts of inbreeding depression in maize. Detailed and precise information on inbreeding in maize was published independently by East in 1908 and by Shull in 1909. Subsequently, studies on other crop plants were reported. It has become clear that in cross-pollinated and in asexually propagated species, inbreeding has harmful effects which are often severe.

Effects of Inbreeding

Inbreeding is generally accompained with a reduction in vigour and reproductive capacity, that is, fertility. There is a general reduction in the size of various plant parts and in yield. In many species, harmful recessive alleles appear after selfing ; plants or lines carrying them usually do not survive. The effects of inbreeding may be summarised as under.

1. **Appearance of Lethal and Sublethal Alleles.** Inbreeding results in the appearance of lethal (leading to death), sublethal and subvital (reducing survival and reproduction rate) characteristics. Such characteristics include : chlorophyll deficiencies, *e.g.*, albina, chlorina etc., rootless seedlings, defects in flower structure etc. Generally, plants carrying such characteristics cannot be maintained and are lost from the population.

2. **Reduction in Vigour.** There is a general reduction in the vigour of the population. Plants become shorter and weaker because of a general reduction in the size of various plant parts.

3. **Reduction in Reproductive Ability.** The reproductive ability of the population decreases rapidly. Many lines (plant progenies) reproduce so poorly that they cannot be maintained. In most of the crops, only a small number of inbred lines have enough fertility to be easily maintained and to be useful in breeding programmes.

4. **Separation of the Population into Distinct Lines.** The population rapidly separates into phenotypically distinct lines. This is because of an increase in homozygosity due to which there is a

random fixation of various alleles in different lines. Therefore, the lines differ in their genotype and, consequently. in phenotype. This leads to an increase in the variance of the population as a whole.

5. **Increase in Homogygosity**. Each line becomes increasingly homozygous following inbreeding. Consequently, the variation within a line decreases rapidly. Ultimately, after 7 to 8 generations of selfing, the lines become almost uniform, since they approach complete homozygosity (>99 per cent homozygosity). *The lines which are almost homozygous due to continued inbreeding and are maintained through close inbreeding are known as inbred lines.* Inbred lines have to be maintained by strict inbreeding, preferably by selfing, in order to keep them homozygous.

6. **Reduction in Yield**. Inbreeding generally leads to a loss in yield. The inbred lines that survive yield much less than the open-pollinated varieties from which they are derived. In maize, the best inbred lines yield about half as much as the open-pollinated varieties from which they were produced. In alfalfa and carrot, the reduction is much greater, while in onions and many cucurbits the reduction in yield is very small.

Degrees of Inbreeding Depression

The various plant species differ considerably in their response to inbreeding. Inbreeding depression may range from very high to very low or it may even be absent. The inbreeding depression observed in various plant species may be grouped into four broad categories : (1) high inbreeding depression, (2) moderate inbreeding depression, (3) low inbreeding depression, and (4) absence of inbreeding depression.

High Inbreeding Depression. Several plant species, *e.g.*, alfalfa (*M. Sativa*), carrot (*D. carota*), hayfield tarweed etc., show very high inbreeding depression. A large proportion of plants produced by selfing show lethal characteristics and do not survive. The loss in vigour and fertility is so great that very few lines can be maintained after 3 or 4 generations of inbreeding. The lines that do survive show greatly reduced yields, generally less than 25 per cent of the yield of open-pollinated varieties.

Moderate Inbreeding Depression. Many crop species, such as, maize (*Z. mays*), jowar (*S. bicolor*), bajra (*P. americanum*) etc., show moderate inbreeding depression. Many lethal and sublethal types appear in the selfed progeny, but a substantial proportion of the population can be maintained under self-pollination. There is appreciable reduction in fertility and many lines reproduce so poorly that they are lost. However, a large number of inbred lines can be obtained which yield upto 50 per cent of the open-pollinated varieties. Production and maintenance of inbred lines are relatively easier in these species than in those showing a high degree of inbreeding.

Low inbreeding Depression. Several crop plants, *e.g.*, onion (*A. cepa*), many cucurbits, rye (*S. cereale*), sunflower (*H. annuus*), hemp (*C. savita*), timothy grass etc., show a small degree of inbreeding depression. Only a small proportion of the plants show lethal or subvital characteristics. The loss in vigour and fertility is small ; rarely a line cannot be maintained due to poor fertility. The reduction in yield due to inbreeding is small or absent. Some of the inbred lines may yield as much as the open-pollinated varieties from which they were developed.

No Inbreeding Depression. The self-pollinated species do not show inbreeding depression, although they do show heterosis (Chapter 17). It is because these species reproduce by self-fertilization and, as a result, have developed *homozygous balance* In contrast, the cross-pollinated species exhibit *heterozygous balance.*

Homogzygous and Heterozygous Balance

The concepts of homozygous and heterozygous balance were advanced by Mather to explain the varied responses of different species to inbreeding. The species that reproduce by cross fertilization are highly heterozygous. These species carry a large number of lethal, subvital and other unfavourable recessive genes, which are of little immediate value to the species. *The sum total of these unfavourable genes is known as genetic load.* The harmful effects of the recessive alleles are masked by their dominant alleles as a result of which they are retained in the population. The population, therefore, develops a genetic organisation which favours heterozygosity. This type of genetic organisation is known as *heterozygous balance,* because it promotes heterozygosity.

The self-fertilized species are naturally homozygous. They have no genetic load because unfavourable recessive genes become homozygous and are eliminated from the population. These species, therefore, develop a genetic organisation which is adapted to homozygosity, *i.e.*, which does not produce undesirable effects in the homozygous state. This type of genetic organisation is known as *homogygous balance.* The self-pollinated species have evolved from cross-fertilized species. It is *suggested that the self-fertilized species retain sufficient heterozygous balance to show the beneficial effects of outcrossing, i.e., heterosis.* The cross-fertilized species that are generally grown in very small populations, *e.g.*, cucurbits, would show some degree of homozygosity due to inbreeding. This would lead to the development of homozygous balance in such cross-fertilized species. As a result, these species would show little or no depression due to inbreeding.

The homozygous and heterozygous balances are concepts of genetic organisation of populations These concepts are neither very clear nor very specific in terms of the physical basis of genetic organisation or the types of gene combinations involved. But it may be visualised that in self-pollinated spicies, those gene combinations

would be favoured which show no injurious effects in the homozygous state. However, upon outcrossing, these gene combinations may show heterosis depending upon the specific gene combinations involved. In the cross-fertilized species, on the other hand, heterozygosity is the natural condition. Therefore, gene combinations that would be deleterious in the homozygous state are not selected against and are maintained in the population. Consequently, such species would show inbreeding depression. The degree of inbreeding depression in these species would be largely determined by the degree of self-fertilization occurring in the natural populations of the species. Cross-fertilized species like maize (*Z. mays*) show upto 10 per cent self-pollination and moderate inbreeding depression. Crops like alfalfa (*M. sativa*) show very little inbreeding and, as a result, a very severe inbreeding depression. Therefore, the homozygous and heterozygous balances may be visualised as gene combinations that are adapted to heterozygosity and homozygosity, respectively.

HETEROSIS

The term heterosis was first used by Shull in 1914. *Heterosis may be defined as the superiority of an F_1 hybrid over both its parents in terms of yield or some other character.* Generally, heterosis is manifested as an increase in vigour, size, growth rate, yield or some other characteristic. But in some cases, the hybrid may be inferior to the weaker parent. This is also regarded as heterosis; we shall focus on this aspect a little later. Often the superiority of F_1 is estimated over the average of the two parents, or the mid-parent. If the hybrid is superior to the mid-parent, it is regarded as heterosis (*average heterosis*). This practice has found some acceptance, particularly in theoretical studies. However, in practical plant breeding, superiority of the F_1 over the mid-parent is of no use since it does not offer the hybrid any advantage over the better parent. Therefore, average heterosis is of little or no use to the plant breeder. More generally, heterosis is estimated over the superior parent ; such an estimate is sometimes referred to as *heterobeltiosis*. The term heterobeltiosis is not commonly used since most breeders regard this to be the only case of heterosis and refer to it as such. However, the commercial usefulness of a hybrid would primarily depend on its performance in comparison to the best commercial variety of the concerned crop species. In many cases, the superior parent of the hybrid may be inferior to the best commercial variety. In such cases, it will be desirable to estimate heterosis in relation to the best commercial variety of the crop ; such an estimate is known as *economic* or *useful heterosis*. Economic heterosis is the only estimate of heterosis which is of commercial or practical value.

In 1944, Powers suggested that the term heterosis should be used only when the hybrid is either superior or inferior to both the parents. Other situations should be regarded as partial or complete dominance. This is easily explained with the help of Table 10.1.

Table 10.1. Heterosis and dominance in relation to parental values.

Position and value of the parents	*Value of the F_1 hybrid*	*Phenomenon*
	>10	Heterosis
Parent A(10)	10	Complete dominance
	<10 but >8	Partial dominance
(Mid-parent)	8	No dominance
	< 8 but >6	Partial dominance
Parent B (6)	6	Complete dominance
	< 6	Heterosis

There has been considerable confusion due to the use of various terms to describe heterosis, and also due to the use of heterosis in different contexts. For clarity and simplicity, it is desirable to avoid the use of many terms to describe similar but somewhat different situations. Two terms, heterosis or hybrid vigour and luxuriance deserve a brief consideration in this context.

Heterosis or Hybrid vigour. Hybrid vigour has been used as a synonym of heterosis. It is generally agreed that hybrid vigour describes only the superiority of hybrids over their parents, while heterosis describes other situations as well. But a vast majority of the cases of heterosis are the cases of superiority of hybrids over the parents. The few cases where F_1 hybrids are inferior to their parents may also be regarded as the cases of hybrid vigour in the negative direction. For example, many F_1 hybrids in tomato are earlier than the parents. Earliness in many crops is agriculturally desirable. It may be argued that the earliness of F_1 hybrids exhibits a faster development in them so that their vegetative phase is replaced by the reproductive phase more quickly than in their parents. *Therefore, the use of heterosis and hybrid vigour as synonyms seems reasonably justified.*

Luxuriance. Luxuriance is the increased vigour and size of interspecific hybrids. The principal difference between heterosis and luxuriance lies in the reproductive ability of the hybrids. *Heterosis is accompanied with an increased fertility, while luxuriance is expressed by interspecific hybrids that are generally sterile or poorly fertile.*

Historical

Hybrid vigour in artificial tobacco (*Nicotiana sp.*) hybrids was first reported by Koelreuter in 1673. Subsequently, many workers reported hybrid vigour in a large number of plant species. These hybrids were produced from interspecific as well as intraspecific crosses. In 1876, Darwin concluded that hybrids from unrelated plant types are highly vigorous. Most of our present knowledge on heterosis comes from work on maize (*Z. Mays*). Maize is perhaps the most extensively studied crop species with respect to heterosis and inbreeding depression. Beal studied the performance of intervarietal hybirds between 1877 and 1882. He reported that some F_1 hybrids

yielded as much as 40 per cent more than the parental varieties. From subsequent studies on intervarietal crosses in maize, it became clear that some of the hybrids showed herterosis, while others did not. Crosses between distinct types, *i.e.*, genetically diverse varieties, exhibited greater heterosis than those involving closely related varieties.

The commercial maize hybrids, are produced by crossing inbred lines rather than open-polinated varieties. This technique has been successfully used in many cross-pollinated species, *e.g.*, bajra, jowar etc. The development of hybrid maize is based on the work of East and Shull beginning in the first decade of the present century. Hybridization between inbreds developed from the same variety or from closely related varieties produces only a small degree of heterosis. In any case, the hybrids from such inbreds are not superior to the open-pollinated varieties from which they were isolated. But hybrids from unrelated inbreds generally show much greater heterosis. Certain inbreds consistently produce better hybrids than others. Further, certain cross combinations are superior to other cross combinations.

Heterosis in Cross- and Self-Pollinated Species

In general, cross-pollinated species show heterosis, particularly when inbred lines are used as parents. In many cross-pollinated species, heterosis has been commercially exploited, for example, in maize, bajra, jowar, cotton (*Gossypium* sp.), sunflower (*H. annuus*), onion (*A. cepa*), alalfa (*M. sativa*) etc. Many crosses in self-pollinated species also show heterosis, but the magnitude of heterosis is generally smaller than that in the case of cross-pollinated species. But in some self-pollinated crops heterosis is large enough to be used for the production of hybrid varieties. Hybrid varieties are commercially used in some vegetables, such as tomato (*L. esculentum*), where a single fruit produces a large number of seeds. *The chief drawback in the use of hybrid varieties in self-pollinated crops is not the absence of sufficient heterosis, but the great difficulty in production of large quantities of hybrid seed.*

Manifestations of Heterosis

Heterosis is the superiority of hybrid over its parents. This superiority may be in yield, quality, disease and insect resistance, adaptability, general size or the size of specific parts, growth rate, enzyme activity etc. These various manifestations of heterosis may be summarised as follows.

1. **Increased yield.** Heterosis is generally expressed as an increase in yield of hybrids. Commercially, this phenomenon is of the greatest importance since higher yields are the most important objective of plant breeding. The yield may be measured in terms of grain, fruit, seed, leaf, tubers or the whole plant.

2. **Increased Reproductive Ability.** The hybrids exhibiting heterosis

show an increase in fertility or reproductive ability. This is often expressed as higher yield of seeds or fruits or other propagules, *e.g.*, tuber in potato (*S. tuberosum*), stem in sugarcane (*S. officinarum*) etc.

3. **Increase in size and General Vigour.** The hybrids are generally more vigorous, *i.e.*, healthier and faster growing and larger in size than their parents. The increase in size is usually a result of an increase in the number and size of cells in various plant parts. For example, fruit size in tomato, head size in cabbage (*B. oleracea*), cob size in maize, head size in jowar (Fig. 10.1.) etc.

4 **Better Quality.** In many cases, hybrids show improved quality. This may or may not be accompanied by higher yields. For example, many hybrids in onion show better keeping quality, but not yield, than open-pollinated varieties.

5. **Earlier Flowering and Maturity.** In many cases, hybrids are earlier in flowering and maturity than the parents. This may sometimes be associated with a lower total plant weight. But earliness is highly desirable in many situations, particularly in vegetables. Many tomato hybrids are earlier than their parents.

6. **Greater Resistance to Diseases And Pests.** Some hybrids are known to exhibit a greater resistance to insects or diseases than their parents.

7. **Greater Adaptability.** Hybrids are generally more adapted to environmental changes than inbreds. In general, the variance of hybrids is significantly smaller than that of inbreds. This shows that hybrids are more adapted to environmental variations. In fact, it is one of the physiological explanations offered for heterosis.

8. In some cases, hybrids show a faster growth rate than their parents. But the total plant size of the hybrids may be comparable to that of the parents. In such cases, a faster growth rate is not associated with a larger size.

9. In some cases, there is an increase in the number of nodes, leaves and other plant parts, but the total plant size may not be larger. Such hybrids are known in beans (*P. vulgaris*) and some other crops.

These are some of the characteristics in which heterosis is easily observed. Many other characters are also affected by heterosis, *e.g.*, enzyme activities, cell division, vitamin content (vit. C content in tomato), other biochemical characteristics etc., but they are not so readily observable.

GENETIC BASES OF HETEROSIS AND INBREEDING DEPRESSION

Heterosis and inbreeding depression are closely related phenomena. In fact, they may be regarded as the two opposite sides of the same coin. The genetic theories that explain heterosis also explain inbreeding depression. There are two main theories to explain heterosis, and consequently, inbreedings depression : (1) dominance hypothesis, and (2) overdominance hypothesis.

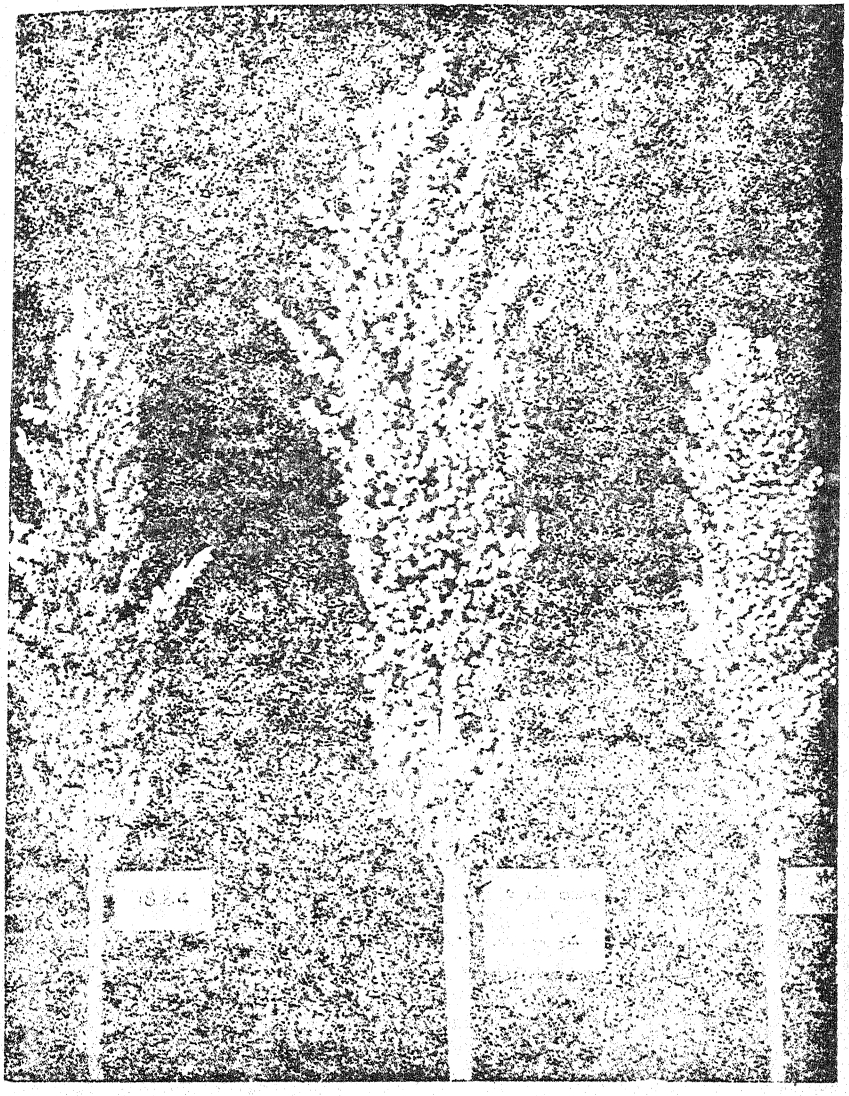


Fig. 10.1. Heads of CSH 1 (in the centee) and its parents IS 84 (on the left) and msCK 60 (on the right). Note the larger head and grain size of the hybrid as compared to its parents.

Dominance Hypothesis

The dominance hypothesis was first proposed by Davenport in 1908. It was later expanded by Bruce, and by Keeble and Pellew in 1910. In simplest terms, this hypothesis suggests that at each locus the dominant allele has favourable effect, while the recessive allele has unfavourable effect. In heterozygous state, the deleterious effects of the recessive alleles are masked by their dominant alleles. *Thus hetersosis results from the masking of harmful effects of recessive alleles by their dominant alleles. Inbreeding depression, on the other hand, is produced by the harmful effects of recessive alleles which become homoyzgous due to inbreeding.* Therefore, according to the dominance hypothesis, heterosis is not the result of heterozygosity ; it is the result of the prevention of expression of harmful recessives by their dominant alleles. Similarly, inbreeding depression does not result from homozygosity *per se*, but from the homozygosity of recessive alleles which have harmful effects.

This hypothesis may be further explained as follows. In open-pollinated populations, plants are highly heterozygous. As a result, they do not show the harmful effects of the large number of recessive alleles present in the population. Inbreeding increases homozygosity. As a result, many recessive alleles become homozygous. Lethal recessive alleles are eliminated by natural selection. But recessive alleles with smaller harmful effects survive in the homozygous condition. Consequently, such alleles reduce the vigour and fertility of the lines that carry them. Inbred lines are nearly homozygous, and different inbred lines would receive different proportions of dominant and recessive alleles. Therefore, different inbred lines may be expected to vary in vigour and yeild. Thus it should be possible to isolate inbreds with all the dominant alleles present in the population. Such inbreds would be as vigorous as the open-pollinated varieties, or even more so. But such inbreds have not been isolated so far.

Similarly, heterosis in an F_1 hybrid is a result of the masking of harmful effects of recessive alleles present in one parent by the dominant alleles present in the other parent and *vice versa*. Obviously, heterosis would depend upon the genotypes of the two parents. Hybrids from parents with similar recessive and dominant alleles would show little or no heterosis (Fig. 10.2, cross I), while those with different alleles would show heterosis (Fig. 10.2, cross II). In practice, some parents produce heterotic progeny, while others do not. Generally, parents of diverse origin are more likely to produce heterotic progeny than those of similar origin.

Objections. Two objections have been raised against the dominance hypothesis. The *first* objection relates to the failure in isolation of lines homozygous for all the dominant genes. The *second* objection is directed at the symmetrical distribution in F_2.

1. Failure in the Isolation of Inbreds as Vigorous as Hybrids. According to the dominance hypothesis, it should be possible to

isolate inbreds with all the dominant genes. Such inbreds would be as vigorous as the F_1 hybrids. However, such inbreds have not been isolated.

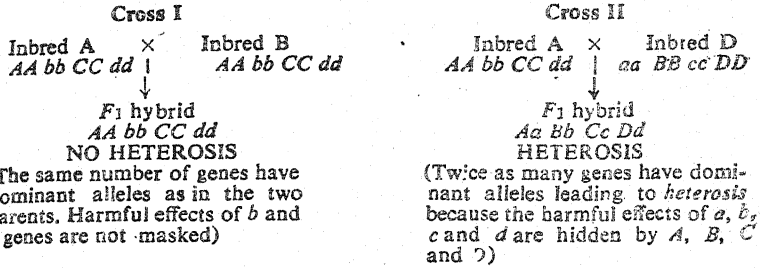


Fig. 10.2. Heterosis as a function of genotypes of the two parents (dominance hypothesis).

2. Symmetrical Distribution in F_2. In F_2, dominant and recessive characters segregate in the ratio of 3 : 1. Quantitative characters, according to the dominance hypothesis, therefore should not show a symmetrical distribution in F_2. This is because dominant and recessive phenotypes would segregate in the proportion $(\frac{3}{4}+\frac{1}{4})^n$, where n is the number of genes segregating. However, F_2s nearly always show a symmetrical distribution.

Explanations for the Two Objections. In 1917, Jones suggested that since quantitative characters are governed by many genes, they are likely to show linkage. It may be expected that dominant and recessive genes governing a character would be linked together (Chapter 9 ; storage of variability). In such a case, inbreds containing all the dominant genes cannot be isolated because this would require several precisely placed crossovers. It would also explain the symmetrical curves in F_2. This is often known as the *Dominance of Linked Genes Hypothesis.*

Later in 1921, Collins showed that if the number of genes governing a quantitative character was large, symmetrical distribution would be obtained even without linkage. Further, it is unlikely that a plant containing all the dominant genes would be recovered if the number of genes were large even if they were not linked. The distribution curve would further become symmetrical due to the effects of environment, that is, due to less than 100 per cent heritability.

Overdominance Hypothesis

This hypothesis was independently proposed by East and Shull in 1908. This is sometimes known as *single gene heterosis, superdominance, cumulative action of divergent alleles,* and *stimulation of divergent alleles. According to this hypothesis, heterozygotes at atleast some of the loci are superior to both the relevant homozygotes.* Thus heterozygote *Aa* would be surperior to both the homozygotes

AA and aa. Consequently, heterozygosity is essential for and is the cause of heterosis, while homozygosity resulting from inbreeding produces inbreeding depression. It would, therefore, be impossible to isolate inbreds as vigorous as F_1 hybrids.

In 1936, East proposed that at each locus there are several alleles, *e.g.*, a_1, a_2, a_3, a_4.....etc., with increasingly different functions. Heterozygotes for more divergent alleles would be more heterotic than those involving less divergent ones. For example, a_1a_4 would be superior to a_1a_2, a_2a_3 or a_3a_4. It is assumed that the different alleles perform somewhat different functions. The hybrid is, therefore, able to perform the functions of both the alleles, which is not possible in the case of the two homozygotes.

Evidence for Overdominance. There are not many clear-cut cases where the heterozygote is superior to the two homozygotes. This has been the biggest objection to the general acceptance of the overdominance hypothesis. But there is no doubt that in the case of some genes, heterozygotes are superior to the homozygotes. In case of maize (*Z. mays*), gene *ma* affects maturity. The heterozygote *Ma ma* is more vigorous and later in anthesis and maturity than the homozygotes *Ma Ma* and *ma ma*. Gustafsson has reported two chlorophyll mutants in barley (*H. vulgare*) that produce larger and more number of seeds in the heterozygous state than the normal homozygotes. Similarly, heterozygotes for the hooded gene in barley show a higher rate of photosynthesis than the two homozygotes.

In human beings (*Homo sapiens*), sickle cell anemia is produced by a recessive gene *s*, which is lethal in the homozygous state. In Africa, the heterozygotes *Ss* are at a selective advantage over the normal *SS* individuals because they are more resistant to malaria. Another case of heterozygote advantage is reported in *Neurospora crassa* (bread mould). Gene *pab* if concerned with the synthesis of *p*-aminobenzoic acid. The heterozygote pab^+ *pab* is more vigorous and shows a faster growth rate than the two homozygotes *pab pab* and pab^+pab^+.

But the number of such genes where heterozygote superiority has been established beyond doubt is limited. There is a large number of cases, however, where heterozygotes for chromosome segments, *e.g.*, inversions etc., or complex loci are known to be superior to the homozygotes. However, the *superiority of heterozygotes need not be a result of overdominance. It could more easily be due to linkages in the repulsion phase or due to epistatic effects.* (Epistasis is an interaction between two or more nonalleles, *i.e.*, different genes. Overdominance, on the other hand, is an interaction between two alleles.)

Comparison between Dominance and Overdominance Hypotheses. The two hypotheses lead to similar expectations. But they differ from each other with respect to one expectation. The similarities and differences between them are listed below.

Similarities. The two hypotheses have the following similarities.

1. Inbreeding would produce inbreeding depression.
2. Outcrossing would restore vigour and fertility.
3. The degree of heterosis would depend upon the genotypes of the two parents. In general, the greater the genetic diversity between the parents, the higher the heterosis.

Difference. Heterozygotes are superior to the two homozygotes according to the overdominance hypothesis, while according to the dominance hypothesis they are as good as the dominant homozygote. Therefore, inbreds as vigorous as the F_1 hybrid can be isolated according to the dominance hypothesis, but it will be impossible according to the overdominance hypothesis.

In spite of the large experimental evidence accumulated, it is not possible to conclusively accept or reject one of the two hypotheses. There are definitely some genes that show heterozygote superiority. But the number of such genes appears to be rather small, and even these cases could be due to linkage in repulsion phase or epistasis or both. *It is generally accepted that heterosis, to a large extent, is due to dominance gene action, but epistasis and overdominance are also involved.* The relative importance of these phenomena is not clearly understood. Recent evidence accumulated with maize seems to suggest that overdominance may not be the primary cause of heterosis. Overdominance is easily imitated by epistasis and linkage, and that most reported cases of overdominance may not represent true overdominance.

PHYSIOLOGICAL BASIS OF HETEROSIS

Earlier studies on the physiological basis of heterosis related to embryo and seed sizes, growth rates in the various stages of development, rates of reproduction and of various assimilation activities. It was suggested that hybrid vigour resulted from large embryo and endosperm size of the hybrid seeds as compared to those of the inbreds. As a result, the rate of growth in the seedling stage may be expected to be greater in the hybrids than in the inbreds. But these relationships were clearly demonstrated in some cases, while in other cases they were not detectable. There is evidence that increased size of hybrids is a result of an increase both in the size and the number of cells. This and other observations indicate a basic difference in the metabolic activities of hybrids and inbreds. In 1952, Whaley concluded that the primary heterotic effect concerns growth regulators and enzymes. He suggested that the hybrid embryo would be able to mobilize storted food materials earlier than those of the inbreds due to a more efficient enzyme system. This would lead to the superiority of hybrids in the early seedling stages.

Most of these studies concerned with the gross expressions of heterosis, *e.g.*, growth rate, cell number and size etc., and their conclusions about the physiological causes of these expressions were highly speculative. There was no evidence to point out clearly the possible reasons for heterozygote advantage. Recent studies demonstrate that heterozygote superiority may be produced in several different ways which may differ considerably from one case to another. These mechanisms are briefly described below.

Reduced Amount of a Single Gene Product. In some cases, the heterozygote produces an intermediate amount of a gene product which may lead to the increased vigour and growth rate. Such a situation would be encountered when the homozygote for the dominant allele produces more and the homozygote for the recessive allele produces less than the optimum level of a product. An example of such a situation is found in the bread mould *Neurospora crassa*. Gene *pab* is responsible for the production of *p*-aminobenzoic acid. The heterozygote pab^{+} *pab* produces intermediate amounts of *p*-aminobenzoic acid and is faster in growth that the two homozygotes, that is, $pab^{+}pab^{+}$ and *pab pab*.

Separate Gene Products. The two alleles may produce proteins with slightly different functions and properties. The heterozygote would possess the proteins produced by both the alleles, while the homozygotes would have only one of the two proteins. Thus the heterozygotes may be expected to be more adapted to changes in the environment. Consequently, they would be superior to the two homozygotes. An example of such a situation is the sickle cell anemia in human beings (*H. sapiens*). The homozygote *ss* does not survive because the defective haemoglobin β^{S} is unable to perform the functions of the normal haemoglobin β^{A}. The heterozygote *Ss* is more resistant to malaria than the normal homozygote *SS*. This is because the defective haemoglobin β^{S} produced by the recessive allele *s* is not suitable for the multiplication of the malarial parasite, which multiplies in the red blood cells. Thus the heterozygotes survive due to the normal haemoglobin β^{A} produced by the normal allele *S*, and are resistant to the malarial parasite due to the defective haemoglobin β^{S} produced by the recessive allele.

Combined Gene Product. This is also known as the hybrid product. Many enzymes are dimers or polymers, that is, they are made of two or more identical polypeptides. In such cases, products of the two alleles in the heterozygote combine to produce an enzyme molecule with somewhat different properties as compared to the enzymes found in the two homozygotes. This new molecule is often detectable by electrophoresis in the heterozygote, and is often termed as the *hybrid substance*. Such enzyme molecules from the heterozygotes may often differ from the enzymes found in the homozygotes in their physical properties, *e.g.*, heat sensitivity. The two alleles governing the enzyme octanyl dehydrogenase-I produce heat sensitive enzymes

in the homozygous state. The enzyme from the heterozygote, however, is less heat sensitive than the enzymes produced by the two homozygotes.

Effects in Two Different Tissues. In some cases, heterozygote superiority may arise in a somewhat strange manner. The two alleles in the homozygous state may produce high levels of an enzyme in two different tissues. The heterozygote may show intermediate levels of the enzyme in both the tissues. In maize, allele Adh^F produces more activity of the enzyme alcohol dehydrogenase in the scutellar tissue of seeds, while allele Adh^S produces more activity in the pollen grains. The hybrid exhibits intermediate activities in both the tissues.

These are some of the known mechanisms that may lead to heterozygote superiority. There may be several yet undiscovered mechanisms responsible for heterosis.

COMMERCIAL APPLICATIONS

Heterosis is observed in almost every crop species studied. Often the degree of heterosis is considerably high to permit its commercial exploitation. Heterosis is commercially used in the form of hybrid or synthetic varieties. Such varieties have been most commonly used in cross-pollinated and often cross-pollinated crop species. In several self-pollinated species also, hybrid varieties have been commercially used. Attempts have been made and are being made to utilize heterosis in breeding of wheat and barley. But so far, these attempts have not been successful. The chief difficulty in commercial exploitation of heterosis is in the production of large quantities of hybrid seed. This is particularly difficult in those self-pollinated species where the number of seeds per fruit is small, *e.g.*, cereals. Some of the species where heterosis has been commercially exploited are listed below.

Crop species	: Asexually propagated species, cross-pollinated species, *e.g.*, maize, jowar, bajra, sugarbeets, sunflower, forage grasses, forage legumes, and cotton, and some self-pollinated crops, *e.g.*, rice (in China).
Vegetable crops	: Tomato, brinjal (*Solanum melongena*), onion, cucurbits, Brussels sprouts etc.
Fruit Trees	: In almost all the fruit trees.
Animals	: Silk worm, poultry, cattle, swine.

This list is by no means comprehensive and many other examples may be found in the literature. In India, heterosis has been commercially exploited in maize, jowar, bajra, cotton, asexually propagated crop species and fruit trees.

SUMMARY

In cross-pollinated species, inbreeding leads to loss in vigour and fertility ; this is known as *inbreeding depression*. There is a general reduction in size of various parts and in yield. In many species, harmful recessive alleles appear in varying frequencies. Many lines may be lost due to severe reduction in vigour and fertility. The degree of inbreeding depression varies considerably from one species to another. Some species, onion, cucurbits etc., show little or no inbreeding depression ; in species like maize and bajra there is moderate inbreeding depression, while in some species, such as alfalfa and carrot, the inbreeding depression is very severe. Self-pollinated species, on the other hand, are adapted to inbreeding and do not show any inbreeding depression.

F_1 hybrids from two unrelated genotypes are often more vigorous and fertile than the parents ; this is known as *heterosis*. Heterosis is generally expressed as an increase in size and yield, but it may be expressed in several other ways, *e.g.*, earlier or later flowering and maturity, greater adaptability, faster growth rate etc. Heterosis is observed both in cross- and self-pollinated species. There are two genetic hypotheses to explain heterosis : dominance hypothesis and overdominance hypothesis. *Dominance hypothesis* states that heterosis is the result of masking of harmful effects of recessive alleles by their dominant alleles. According to *overdominance hypothesis*, heterosis is the result of superiority of the heterozygotes. There is no conclusive evidence to support or to reject either of the two hypotheses. Heterozygotes at certain loci are superior to homozygotes. *It is generally believed that heterosis is the result of dominance and epistatic interactions ; some genes may also show overdominance.*

At the biochemical level, heterosis may result in one of many ways. Some of the mechanisms known so far are : reduced amount of a single gene product, separate gene products by the two alleles, combined gene product or the hybrid substance and effects in two different tissues. Heterosis is commercially exploited in form of hybrid varieties or synthetic varieties. Heterosis has been extensively used in many crop species, vegetables, fruit trees and in animals.

QUESTIONS

1. Define inbreeding depression and hybrid vigour. How do these phenomena affect the self- and cross-pollinated species ? Explain with the help of suitable examples.
2. Differentiate between the following ; (i) homozygous and homogeneous lines, (ii) homozygous and heterozygous balance, (iii) genetic load and gene pool, (iv) heterosis and luxuriance, (v) heterosis and hybrid vigour, (vi) dominance and overdominance.
3. What are the main features of inbreeding depressipn ? Briefly describe the degrees of inbreeding depression encountered in crop species.
4. Define heterosis. List its characteristic features, applications and achievements in crop improvement.
5. Explain the dominance and overdominance hypotheses of heterosis giving their main features, objections and answers to the objections. Which of the above two hypotheses is more widely accepted and why ?
6. Write short notes on the following : (i) sickle cell anaemia, (ii) *pab* locus in *Neurospora*, (iii) *ma* gene in maize, (iv) *Adh* alleles in maize, (v) inbreeding depression, and (vi) hybrid vigour.
7. Discuss the physiological bases of heterosis.

Suggested Further Reading

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CHAPTER 11

Mass Selection

In mass selection a large number of plants of similar phenotype are selected and their seeds are mixed together to constitute a new variety. The plants are selected on the basis of their appearance or phenotype. Therefore, selection is done for easily observable characters like plant height, ear type, grain colour, grain size, disease resistance, tillering ability, lodging resistance, shattering resistance etc. Sometimes, yield of the plant may be used as a criterion of selection. If the population has variation for grain characteristics like seed colour and seed size, selection may be done for them before the seeds of selected plants are mixed together.

The population obtained from the selected plants would be more uniform than the original population. It may be expected to be more or less uniform for easily observable characters, like, plant height, seed colour, presence of awns etc., governed by one or few major genes. However, the population is likely to show a considerable variation for quantitative characteristics like yield, quality, adaptability etc. The original population would have been a mixture of several purelines, and the plants selected from it would be homozygous. Since several plants are selected and their seeds are mixed, the selected population would be a mixture of several similar looking purelines. Thus a variety developed through mass selection would have considerable genetic variation, and consequently further mass selection or pureline selection may be done in such a variety at a later date.

Generally, the plants selected in mass selection are not subjected to progeny test. But Allard (1960) maintains that progeny test should be done. In such a case, poor and defective progenies are rejected. The remaining progenies are mixed together to constitute the new variety.

APPLICATIONS OF MASS SELECTION

In case of self-pollinated crops, mass selection has two major

applications : (1) improvement of *Desi* or local varieties, and (2) purification of the existing pureline varieties.

Improvement of Local Varieties. Mass selection is useful for the improvement of land, *desi* or local varieties of self-pollinated crops. The local varieties are mixtures of several genotypes which may differ in flowering or maturity time, disease resistance, plant height etc. Many of these plant types would be inferior and low yeilding. As a result, they would lower the performance of the local variety. Hence the elimination of poor plant types through mass selection would improve the performance and uniformity of the variety. The local varieties have been under cultivation for a long time. Therefore, they would generally be well adapted to the local environment and would be relatively stable in their performance. Mass selection would improve the variety without adversely affecting its adaptability and stability because the new variety would be made up of most of the superior plant types present in the original local variety.

Purification of Existing Pureline Varieties. The other application of mass selection is to maintain the purity of existing pureline varieties. Purelines tend to become variable with time due to mechanical mixtures, natural hybridization and mutation. It is, therefore, necessary that the purity of pureline varieties be maintained through regular mass selection. At present, mass selection is used precisely for this purpose.

Thus as a breeding method mass selection has only a limited application for the improvement of self-pollinated crops. It is generally not used for the handling of segregating populations derived from hybridization. Sometimes, many phenotypically similar purelines differing in characters like disease resistance are mixed together to make-up a new variety. This may be considered as a variation of mass selection. But in this case, the purelines are progeny tested and evaluated for their performance before they are mixed. They may also be evaluated for their performance as a mixture of the purelines. Mass selection has also been used for the selection of desirable traits in segregating populations, *e.g.*, for seed size in oats. But this application is of limited scope where mass selection for the concerned character is rather easy and avoids extensive data collection necessary for single plant selection, *e.g.*, for days to maturity, seed size etc.

However, in breeding of cross-pollinated species, mass selection has been very important. In such crops, inbreeding must be avoided since it leads to a loss in vigour and yield. Since in mass selection several plants are selected and their seeds are mixed together to raise the next generation, inbreeding is avoided or kept to a minimum. Further, because of the heterozygous nature of the population, several cycles of mass selection may effectively be practised.

THE PROCEDURE OF MASS SELECTION

The general procedure for mass selection in developing a new variety is outlined below and schematically presented in Fig. 11.1. But the procedure may be modified by the breeder according to the needs.

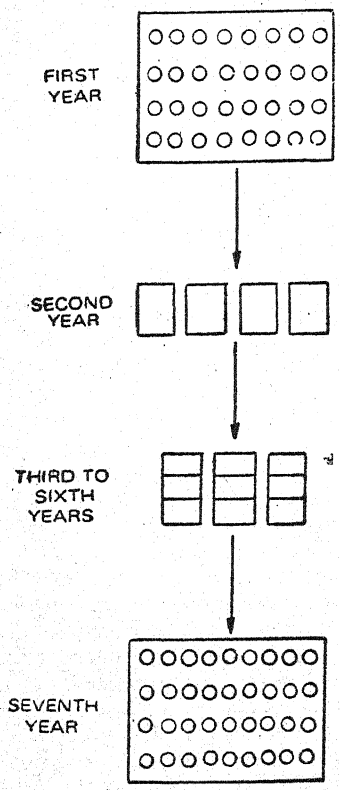


Fig. 11.1. Mass selection in self-pollinated crops as used to develop new varieties. For maintaining the purity of pureline varieties, operations of the first year may be repeated every year or after every few years.

First year. A large number of phenotypically similar plants are selected for their vigour, plant type, disease resistance and other desirable characteristics. The number of plants selected may vary from few hundred to few thousand. If too many plants are selected, the improvement is likely to be small. But if too few plants are selected, the adaptation of the variety may become poor. Seeds from the selected plants are composited to raise the next generation.

Second Year. The composite seed is planted in a preliminary yield trial along with standard varieties as checks. The variety from which the selection was made should also be included as a check to determine if there has been an improvement due to selection. Phenotypic characteristics of the new variety are critically observed.

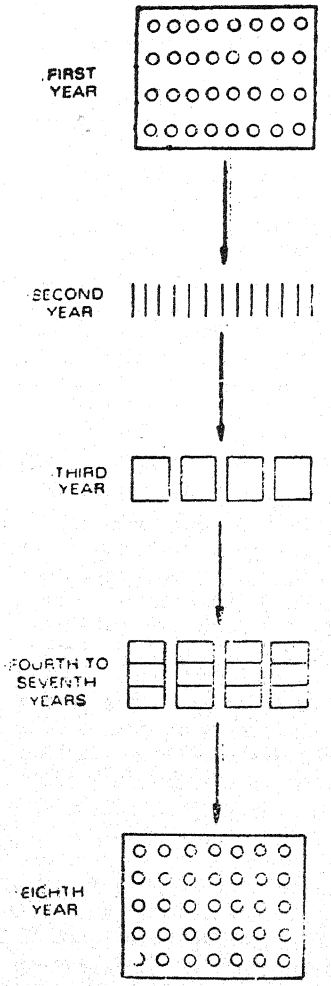


Fig. 11.2. Mass selection in self-pollinated crops coupled with progeny testing. This method is more useful than the one outlined before. It is commonly used for maintaining the purity of pureline varieties.

Third to Sixth Year. The variety is evaluated in coordinated yield trials at several locations. This is done to test the performance of the new variety at different locations within an agroclimatic zone. *First,* the variety is evaluated in an *initial evaluation trial* for one year. If the variety is promising, it is promoted to *uniformity regional trials* for two or more years. If promising, the variety will be identified for release (see chapter 27 for more detail).

Seventh Year. The variety may be released for cultivation if found suitable and if recommended by the Central or State Variety Release Committee.

Two variations of mass selection are given below to serve two different objectives. In *improving a local variety* (Fig. 11.2), a large number of plants are selected and their seeds are harvested separately. The number of plants may range from several hundred to few (2 or 3) thousand. In the second year, individual plant progenies are grown for progeny test. Poor, weak and defective progenies are rejected. Progenies from heterozygous plants are also discarded. Care should be taken not to discard more than 20-25 per cent of the progenies. Seeds from the remaining progenies are mixed together to make-up the new variety. In the third and fourth years, the variety is subjected to yield trials in different environments to check its performance and adaptability. The new variety is quickly released since it would retain the adaptation of the originial variety with a superior performance. Thus prolonged testing is not necessary; this reduces the cost and the time required for developing the new variety.

The second variation of mass selection is used *for the purification of existing pureline varieties.* In the first year, 2-3 hundred or more plants are selected and harvested separately from the pureline to be purified. The plants selected are those typical for the variety. In the second year, progenies from the selected plants are grown and observed critically. Progenies which are different from the typical features of the variety are rejected. The remaining progenies are mixed together to constitute the concerned variety. This process may be repeated every few years to keep the variety pure or as often as it is found necessary. This procedure is followed to produce the basic seed of purline varieties ; the basic seed is used for the production of breeder seed (see chapter 28).

MERITS OF MASS SELECTION

1. Since a large number of plants are selected, the adaptation of the orginal variety is not changed. It is generally accepted that a mixture of closely related purelines is more stable in performance over different environments than a single pureline. Thus varieties developed through mass selection are likely to be more widely adapted than purelines.
2. Often extensive and prolonged yield trials are not necessary. This reduces the time and cost needed for developing a new variety.

3. Mass selection retains considerable genetic variability. Therefore, another mass selection after few years would be effective in improving the variety further.
4. It is a less demanding method. The breeder can devote more time to other breeding programmes.

DEMERITS OF MASS SELECTION

1. The varieties developed through mass selection show variation and are not as uniform as pureline varieties. Therefore, such varieties are generally less liked than pureline varieties.
2. The improvement through mass selection is generally less than that through pureline selection. It is because at least some of the plant progenies which make up the new variety would be poorer than the best pureline that may be selected from among them.
3. In the absence of progeny test, it is not possible to determine if the selected plants are homozygous. Even in self-pollinated species, some degree of cross-pollination does occur. Thus there is some chance that some of the plants may be heterozygous. It is also not known if the phenotypic superiority of the selected plants is due to the environment or the genotype. As suggested by Allard, progeny test may be included in mass selection programmes to overcome this defect.
4. Due to the popularity of pureline varieties, mass selection is not commonly used in the improvement of self-pollinated crops. But it is a quick and convenient method of improving old local varieties in the areas or crop species where crop improvement has just begun.
5. Varieties developed by mass selection are more difficult to identify than purelines in seed certification programmes.
6. Mass selection utilizes the variability already present in a variety or population. Therefore, only those varieties that show genetic variation can be improved through mass selection. Thus mass selection is limited by the fact that it cannot generate variability.

ACHIEVEMENTS

Mass selection, in one form or the other, must have been used by the prehistoric man to develop the present-day crop species. The differentiation of cultivated species from the wild forms clearly shows the efficacy and the contribution of mass selection to crop improvement. Mass selection was extensively used by farmers and agriculturists for the improvement of self-pollinated crops before pureline selection came in to practice. Subsequently, it was completely replaced by pureline selection as a method of crop improvement.

At present, the use of mass selection is limited to the purification of pureline varieties of self-pollinated crops. Mass selection is

routinely practised every year or every few years to maintain the purity of pureline varieties. The contribution of mass selection in this regard would become much more obvious when one considers that pureline varieties tend to become genetically variable in a few years due to mechanical mixture, mutation etc. Thus the superiority of purelines would be lost quickly if their purity was not maintained through mass selection. All the available pureline varieties in self-pollinated crops are maintained through mass selection.

SUMMARY

Mass selection consists of selection of few hundred to few thousand phenotypically superior plants whose seeds are composited without or after progeny testing. The new variety is tested in yield trials before being released. When a large number of plants are selected, prolonged testing is generally not necessary. Mass selection is simple, easy and less demanding. Release of the new variety requires less time and cost than in the case of purelines. The new variety is stable in performance and is widely adapted. Mass selection is used to improve old local varieties or to purify existing varieties or purelines. Progress under mass selection is generally small, the variety developed is not as uniform as a pureline, and when progeny test is not done the breeding value of the selected plants is not known.

QUESTIONS

1. Define mass selection. Describe the procedure of mass selection and discuss its merits and demerits.
2. Describe the applications of mass selection. Describe the procedure of mass selection as applied for maintaining the genetic purity of pureline varieties.

Suggested Further Reading

ALLARD, R.W. 1960, Principles of Plant Breeding. John Wiley and Sons, Inc, New York.

FREY, K.J. 1967. Mass selection for seed width in oat populations. Euphytica 16 : 341-349.

GEADELMANN, J.L. and FREY, K.J. 1975. Direct and indirect mass selection for grain yield in bulk oat populations. Crop. Sci. 15 : 490-494.

CHAPTER 12

Pureline Selection

A pureline is the progeny of a single, homozygous, self-pollinated plant. As a result, all the individuals within a pureline have identical genotype, and any variation within a pureline is solely due to the environment. In a self-pollinated species, all the plants are expected to be homozygous because of continued self-fertilization. Thus progeny of a single plant from a population of a self-pollinated crop would be a pureline. *In pureline selection, a large number of plants are selected from a self-pollinated crop and are harvested individually; their individual progenies are evaluated, and the best progeny is released as a pureline variety.* Therefore, pureline selection is also known as *individual plant selection.* A *pureline variety* is a variety obtained from a single homozygous plant of a self-pollinated crop. Apparently, a variety developed through pureline selection would be a pureline variety, while that developed through mass selection would be a mixture of several purelines. In self-pollinated crops, pureline varieties are far more common than mixtures of purelines.

CHARACTERISTICS OF PURELINES

1. *All the plants within a pureline have the same genotype* as the plant from which the pureline was derived. This is because the parent plant was homozygous and self-fertilized.

2. *The variation within a pureline is environmental and nonheritable.* Since all the plants within a pureline are of identical genotype, the variation within a pureline is due to the environment only. Therefore, selection within a pureline will generally not be effective.

3. *Purelines become genetically variable with time.* The genetic variation is produced by mechanical mixtures, natural hybridization or mutation. Mechanical mixture and natural hybridization can be prevented by careful handling of purelines. However, mutations would produce genetic variability in due course of time. Man can do nothing about it, except that he may attempt to maintain the purity of purelines through mass selection.

USES OF PURELINES

1. **As A Variety.** A superior pureline may be used as a variety. Almost all the present varieties of self-pollinated crops are purelines.

2. **As Parents in A Hybridization Programme.** A pureline that is not suitable for release as a variety may serve as a parent in the development of new varieties. Hybridization programmes are invariably based on purelines ; some of these purelines may not be suitable for use as commercial varieties.

3. **In Studies on Mutation.** In studies on spontaneous or induced mutations, particularly those affecting quantitative characters, purelines have to be used. This is because any genetic variation arising in a pureline would be due to mutation if care has been taken to avoid mechanical mixture and natural hybridization.

4. **Other Studies.** In many biological investigations, such as, medicine, immunology, physiology, biochemistry, nutrition etc., use of highly inbred lines (virtually purelines) of mice, guinea pigs, rabbits etc. is essential. This is done to avoid genetic variation in the experimental material so that the effects of the treatments are easily detected. And furthermore, the effects of treatment are not confused with those due to genetic variation in the experimental material.

HISTORY OF PURELINE SELECTION

Pureline selection dates back to mid-nineteenth century or even earlier. Between 1840 and 1860, several workers, notably Le Couteur, Shireff, Hallet and Vilmorins, practised individual plant selections in crops like wheat (*T. aestivum*), barley (*H. vulgare*) and sugar beets (*B. vulgaris*). Loui de Vilmorin proposed the *Vilmorin Principle* or *Vilmorin Isolation Principle*, which is the basis for progeny test. The Svalof Experiment Station of the Swedish Seed Association was established in 1806. The pureline selection (or individual plant selection) was refined to its present form by the workers at this station. The genetic basis of purelines was explained subsequently by Johannsen in 1903. Pureline selection has been the most commonly used method of improvement of self-pollinated crops. Almost all the present day varieties of self-pollinated crops are purelines.

APPLICATIONS OF PURELINE SELECTION

Pureline selection has several applications in the improvement of self-pollinated crops. It is used to improve local or *desi* varieties, old pureline varieties and introduced varieties.

Improvement of Local Varieties. Pureline selection is the favourite method for the improvement of local varieties which have considerable genetic variability. A large number of varieties have been

developed by this method. Some examples are NP 4 and NP 52 wheat (*T. aestivum*), NP 11 and NP 12 linseed (*L. usitatissimum*), T 1 cowpea (*V. anguiculata*) and T 1 mung (*V. radiata*) ; these are pureline selections from old local varieties. Pusa Sawani bhindi (okra) is a selection from a variety from Bihar ; it shows an acceptable level of field resistance to yellow mosaic virus.

Pureline Selection in Introduced Varieties. Introduced materials are often subjected to pureline selection to develop suitable varieties. Several varieties have been selected from introduced varieties. Shining Mung 1 is a pureline selection from an introduced variety Kulu Type 1, and PS 16 is a selection from an introduction from Iran. Kalyan Sona is a leaf rust resistant selection from an introduction from CIMMYT, Mexico. For other examples of such an application of pureline selection see Chapter 2.

Improvement of Old Pureline Varieties. Purelines become variable with time. Often selection is used to isolate new varieties from such genetically variable purelines. Some examples of pureline varieties isolated from old pureline are : Chafa (from No. 816) gram; Jalgaon 781 (from China Mung 781), Khargone 1 (from K 119-56), Pusa Baisakhi (from T 44), CO 2 (from PLS 365/3) and CO 3 (from PLS 367) mung ; and CO 2 (from P 160), CO 3 (from PLS 364) and B 76 (from No. 1766) urid (*Vigna mungo*). Sometimes off-type plants appear in purelines. Such off-type plants may be selected to develop new pureline varieties. A dwarf off type was selected from the tall pureline variety of rice Kalimoonch 64, and released as a new pureline variety Shyama.

Selections for A New Characteristic in A Pureline. Sometimes an opportunity for pureline selection arises when there is a need to select for some new character which was not previously important Such a character may be resistance to a disease, plant type, grain type etc. An example of such a selection is the isolation of jowar (*S. bicolor*) variety resistant to root rot caused by *Periconia circinati*. Sorghum variety Dwarf Yellow Milo was not bred for resistance to this disease. In 1926, this disease became widespread in Kansas, U.S.A. Resistant plants were isolated from a root rot infested field to develop a new root rot resistant variety.

Selection in The Segregating Generations from Crosses. Purelines are generally selected from the segregating generations of crosses. Purelines in such cases may be selected using pedigree, bulk or backcross method. These methods will be discussed in more detail in Chapters 13-15.

A GENERAL PROCEDURE FOR PURELINE SELECTION

A general procedure for pureline selection is described below (Fig. 12.1). The number of years required for any step in the procedure and the procedure itself may be modified according to the need. The purpose of this description is to serve as a general guide for the

student. The pureline has three steps : (1) selection of individual plants from a local variety or some other mixed population, (2) visual evaluation of individual plant progenies, and (3) yield trials.

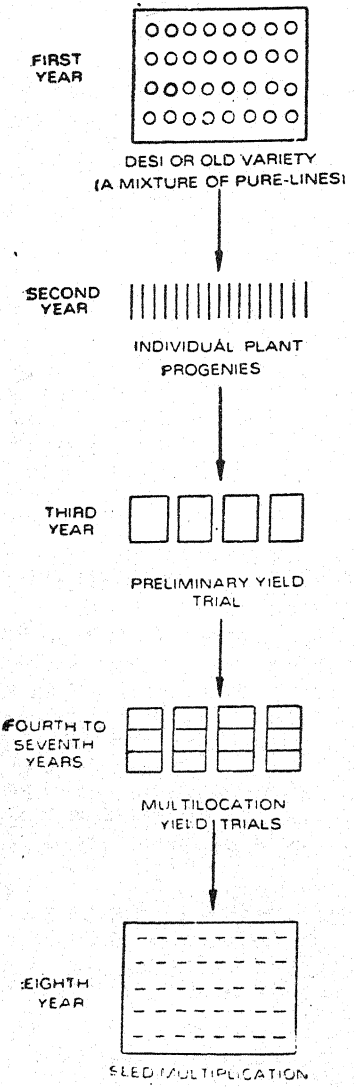


Fig. 12.1. Schematic representation of pureline selection in self-pollinated crops.

Step One

First Year. A large number of plants (200-3000) are selected from a *desi* or local variety or some other mixed population, and their seeds are harvested separately. It is desirable to grow the mixed variety from which selection is to be done, with proper spacing so that individual plants can be observed and evaluated. In case individual plants cannot be identified, individual heads or stems may be selected.

The number of plants to be selected depends upon the breeder's discretion. However, it should be kept in mind that the selected plants will be homozygous. Therefore, all the genetic variation will be between the selected plants, that is, between plant progenies, and there will be no genetic variation within the progenies. Thus it is essential that superior genotypes must be selected from the mixed population. The number of plants selected should be as large as possible in view of the available time, land, funds and labour. When 2-3 thousand plants are selected, they may be picked randomly. But when a smaller number of plants is selected, careful selection for superior types should be made.

In any case, it is advisable to select for easily observable characters, such as flowering and maturity times, disease resistance, presence of awns, plant height etc. If desirable types are not included in the initial selection, they cannot be expected to be present in the progeny.

Step Two

Second Year. Progenies from individual plants are grown separately with proper spacing. The objective of this step is to drastically reduce the number of plant progenies to be carried to step three. This is because step three requires substantial land, labour, time and funds.

The progenies are evaluated visually. Poor, weak and defective progenies are rejected. Segregating progenies are discarded unless they show a great promise. Selection for plant type, plant height, grain type, ear characteristics, flowering and maturity times etc. are made because these characters are generally more simply inherited than yield. Disease epiphytotics may be created to test the progenies for disease resistance. The number of progenies should be reduced as much as possible to facilitate replicated yield trials. The superior progenies are harvested separately. Since each progeny would be a pureline, selection within progenies would be useless.

If necessary, this process may be repeated for one or more years.

Step Three

Third Year. This step consists of a replicated yield trial for a critical

evaluation of the selected progenies. The best variety is used as a check, and should be planted at regular intervals, *e.g.*, after every 20-25 progenies, for ease in comparison. *If enough seed is available a preliminary yield trial may be conducted.*

Selection is made for easily observable characters including disease resistance. In case of a preliminary yield trial, yield of progenies is also used as a criterion of selection. *The main objective is to further reduce the number of progenies.*

Fourth Year. Replicated yield trials are conducted by the breeder using the best available variety as a check. Observations are recorded on disease resistance, flowering and maturity times and other characteristics. Based on these and yield data, superior progenies are identified. Quality tests may be conducted and used as a criterion of selection. Each progeny is equivalent to a strain as it is a pureline. The promising strains are then included in coordinated yield trials for further evaluation of the strains.

Fifth to Eighth Years. The promising strains are evaluated at several locations alongwith strains from other breeders. The best released varieties are used as checks.

Ninth Year. The best progeny or strain is released as a new variety.

The time required in each step may be more or less than that outlined in the above scheme depending upon several factors. For example, if enough seed is available from the second year, preliminary yield trial may be conducted in the third year. This would save one year. Further, evaluation in coordinated yield trials may last more than the 4 years indicated above. This would depend upon the performance of the strain in the multiplication trials.

ADVANTAGES OF PURELINE SELECTION

1. Pureline selection achieves the maximum possible improvement over the original variety. This is because the variety is the best pureline present in the population.
2. Pureline varieties are extremely uniform since all the plants in the variety have the same genotype. Such a uniform variety is more liked by the farmers and the consumers than a less uniform variety developed through mass selection.
3. Due to its extreme uniformity, the variety is easily identified in seed certification programmes.

DISADVANTAGES OF PURELINE SELECTION

1. The varieties developed by pureline selection generally do not have wide adaptation and stability in production possessed by the local or *desi* varieties from which they are developed.

2. The procedure of pureline selection requires more time, space and more expensive yield trials than mass selection.
3. The upper limit on the improvement is set by the genetic variation present in the original population.
4. The breeder has to devote more time to pureline selection than to mass selection. This leaves less time for other breeding programmes.

COMPARISON BETWEEN PURELINE SELECTION AND MASS SELECTION

Mass selection and pureline selection differ in the procedure the product and the improvement gained. The chief differences between the two methods of selection are presented in Table 11.1. It would be seen that the differences between the two methods arise chiefly because of the difference in the number of selected plants included to produce the new variety.

ACHIEVEMENTS

Pureline selection was the most extensively used breeding method in the early days of crop improvement work in India. At that time, genetically variable *desi* or local varieties were available that offerred excellent opportunities for pureline selection. A large number of improved varieties were developed by this method in self-pollinated crops like wheat (*T. aestivum*), barley (*H. vulgare*), rice (*O. sativa*), pulses, *e.g.*, gram (*C. arietinum*), mung (*V. radiata*), urid (*V. mungo*) etc., oilseeds, *e.g.*, groundnut (*A. hypogaea*), linseed (*L. usitatissimum*), self-compatible species of *Brassica, viz.*, rai (*B. juncea*), toria (*B. campestris* var. *toria*), tobacco (*Nicotiana* sp.), cotton (*Gossypium* sp.), jowar (*S. bicolor*), jute (*Corchorus* sp.), many vegetables etc. The examples of varieties developed through pureline selection are too numerous to be enumerated. It may be pointed out that for a long time varieties developed through pureline selection dominated the Indian agriculture. Some of the examples are listed below.

Many wheat varieties developed through pureline selection were very popular and widely cultivated, *e.g.*, NP 4, NP 6, NP 12, Pb 8, Pb 8A, Pb 9 D, Pb 11, C 13, (later K 13), K 46, K 53, K 54 etc. Mung varieties T 1 and B 1 ; rai variety L 18 ; cotton varieties Combodia Coimbatore 2, Gadag 1, MCU 1 ; rice varieties Mtu 1, Mtu 2, Mtu 7, Ar 1, S 155, BR 1, BR 3, Patni 6, Waner, T 1, T 3, T 22, T 29; barley varieties C 251, C 50, K 12 ; tobacco (*N. tabacum*) varieties NP 28, NP 63, NP 70 ; tobacco (*N. rustica*) varieties Harrison Special 9, Chatham etc. are but a few examples of the varieties developed through pureline selection. Mung T 1 was selected from a collection from Muzaffarpur, Bihar, while B 1 was selected from Chaitali Mung. Similarly, urid varieties T 9, T 27,

Naveen, Kulu 4 and T 122 are selections from samples collected from Bareilly, Sindh, Bihar, Kulu Valley and Shillongani, respectively.

Table 12.1. Comparison between pureline and mass selections.

	Pureline selection	*Mass selection*
1.	The new variety is a pureline.	The new variety is a mixture of purelines.
2.	The new variety is highly uniform. In fact, the variation within a pureline variety is purely environmental.	The variety has genetic variation for quantitative characters, although it is relatively uniform in general appearance.
3.	The selected plants are subjected to progeny test.	Progeny test is generally not carried out.
4.	The variety is generally the best pureline present in the original population. The pureline selection brings about the greatest improvement over the original variety.	The variety is inferior to the best pureline because most of the purelines included in it will be inferior to the best pureline.
5.	Generally, a pureline variety is expected to have a narrower adaptation and lower stability in performance than a mixture of purelines.	Usually the variety has a wider adaptation and greater stability than a pureline variety.
6.	The plants are selected for their desirability. It is not necessary that they should have a similar phenotype.	The selected plants have to be similar in phehotype since their seeds are mixed to make up the new variety.
7.	It is more demanding because careful progeny tests and yield trials have to be conducted.	If a large number of plants are selected, extensive yield trials are not necessary. Thus it is less demanding on the breeder.
8.	Generally, 9-10 years are required to develop a new variety.	Generally, 5-7 years are required to develop a new variety.
9.	Selection within a pureline variety is ineffective unless it has become genetically variable.	Selection within a variety developed by mass selection is effective since it has genetic variation.
10.	The produce of a pureline variety is uniform in quality.	The produce is generally not uniform since different purelines making up the variety may differ in the quality of their gains etc.
11.	The variety is easily identified in seed certification programmes.	The variety is relatively difficult to identify in seed certification programmes.
12.	Pureline selection is used in self-pollinated and often cross-pollinated crops.	Mass selection is used in both self- and cross-pollinated crops.

An introduction of *Gossypium hirsutum* cotton from U.S.A. was subjected to mass selection and Dharwar-American variety was isolated. Dharwar-American was improved by pureline selection to isolate Gadag 1 (mass selection in Dharwar-American produced the

variety Dodahatti Local). Gadag 1 was superior to the parent variety Dharwar-American in yield and other agronomic characters, but was susceptible to leaf blight. Another pureline cotton variety Buri 107 was isolated from the Buri variety of *G. hirsutum*. Buri 107 represented improvements in staple length, spinning quality, ginning outturn and was resistant to wilt and frost.

Tobacco (*N. rustica*) variety Harrison Special and culture 40D were introduced from U.S.A. Pureline selection in Harrison Special yielded HS 9 (Harrison Special 9) and Harrison Special varieties (both are similar to each other) which yield about 10 per cent more than the original variety and are more uniform. Chatham was selected from the culture 40D ; it is superior to HS9 in cured leaf colour and is suitable for late planting.

Keliu variety of bidi tobacco was subjected to pureline selection to isolate varieties Keliu 49 (early by 10 days, 12 per cent more yield and better cured leaf colour than Keliu) and Keliu 20 (12 per cent more yield than Keliu 49, cured leaf quality comparable). Other pureline selections in tobacco are Surti 20, Lanka 27, T 23, T 59, Ramol 43 etc.

SUMMARY

Pureline selection consists of selection of a large number of plants whose individual progenies are tested and the best progeny is released as a variety. It is the most common method of developing a variety from local or *desi* varieties, introductions and old pureline varieties, and for the isolation of desirable off-type and disease resistant lines from pureline varieties. Pureline varieties are extremely uniform genotypically and phenotypically, but they become variable with time. Purelines are used as varieties, parents in hybridization programmes and in various biological studies. The pureline varieties are desirable for their uniformity, and they are easily identified in seed certification programmes. Pureline selection achieves the maximum improvement over the original variety. But this method requires more attention from the breeder and a longer time than mass selection in developing a variety. Pureline varieties are also developed from segregating generations of crosses between two or more purelines. In India, a large number of varieties have been developed by the pureline selection.

QUESTIONS

1. Define pureline. List the various characteristics of purlines and their uses.
2. Briefly describe the procedure for pureline selection. Discuss the applications of this method in crop improvement and the achievements through this method.
3. Discuss the merits and demerits of pureline selection. Compare mass selection with pureline selections and explain why pureline selection has been used more widely than mass selection.

Suggested Further Reading

ALLARD, R.W. 1960. Principles of Plant Breeding. John Wiley and Sons, Inc., New York.

CHAPTER 13

Pedigree Method

Mass selection and pureline selection, described in Chapters 11 and 12, are used for the selection of new varieties from mixed populations that have homozygous plants. These methods cannot be applied to segregating populations, *e..g*, F_2, F_3 etc., obtained from crosses. The methods generally used for handling the segregating generations may be grouped into three categories: (1) pedigree method, (2) bulk method, and (3) backcross method. Generally, the objective of these methods is to develop pureline varieties. Occasionally, the purelines developed using these methods may be composited and used as a mixture of purelines.

In the pedigree method, individual plants are selected from F_2 and the subsequent generations, and their progenies are tested. During the entire operation, a record of all the parent-offspring relationships is kept ; this is known as *pedigree record.* Individual plant selection is continued till the progenies show no segregation. At this stage, selection is done among the progenies because there would be no genetic variation within the progenies.

PEDIGREE RECORD

In pedigree method, a detailed record of the relationship between the selected plants and their progenies is maintained. As a result, each progeny in every generation can be traced back to the F_2 plant from which it originated. Such a record is known as *pedigree record* or simply *pedigree. The pedigree may be defined as a description of the ancestors of an individual and it generally goes back to some distant ancestor or ancestors in the past.* Thus a pedigree would describe the parents, grandparents great-grandparents and so on of an individual and, preferably, the blood relatives of the ancestors as well. The pedigree is helpful in finding out if two individuals are related by descent, *i.e.*, have a common parent in their ancestry, and therefore are likely to have some genes in common.

MAINTENANCE OF PEDIGREE RECORD

The pedigree record may be kept in several different ways, but it should be simple and accurate. Generally, each cross is given a number : the first two digits of this number refer to the year in which the cross was made, and the remaining digits denote the serial number of the cross in that year. For example, the number, 7911 denotes the cross number 11 of the year '79. In the segregating generations, one of the following two systems of designation may be followed.

In the *first* system, the individual plant progenies in each generation are assigned row numbers corresponding to their location in the plot. In addition, each progeny in F_4 and the subsequent generations is assigned the row number of the progeny in the previous generation from which it was derived. Thus each F_2 progeny, derived from individual F_2 plants, is given a number corresponding to the row number at which it is located in the F_3 plot. Plants selected from a F_3 progeny are identified by the row number of that progeny. When individual plant progenies are grown in F_4, each progeny is also given the row number of the F_4 plot at which it is located in addition to the F_3 row number. Similarly, the plants selected from a progeny in F_4 are given the row number of that progeny in the F_4 plot. After the F_5 progenies are planted, the row numbers of progenies in the F_5 plot are added to the F_4 row numbers. The same procedure is followed in the subsequent generations as outlined below.

Generation	*Number*	*Description*
F_3	7911-7	Progeny in the 7th row in the F_3 plot.
F_4	7911-7-4	Progeny in the 4th row in the F_4 plot, selected from the progeny in the 7th row of the F_3 plot.
F_5	7911-4-14	Progeny in the 14th row in the F_5 plot, selected from the progeny in the 4th row in the F_4 plot.
F_6	7911-14-3	Progeny in the 3rd row in the F_6 plot, selected from the progeny in the 14th row in the F_5 plot.

Thus each progeny can be traced back to the F_3 progeny (or the F_2 plant) from which it originated. But for determining the pedigre of a progeny, the breeder has to consult the records of the previous years.

In the *second* system, in each generation the selected ptants are assigned serial numbers within individual progenies : each progeny or selected plant bears the serial numbers of all the plants in the previous generations related to it by direct descent. Thus the plants selected in F_2 are given serial numbers ; the F_3 progenies from these plants are given the serial numbers of the parent F_2 plants. The plants selected from a progeny in F_3 are given the number of that

progeny ; in addition, each selected plant form that progeny is also given a serial number. These two numbers make up the progeny number in the F_4 generation. Similarly, the plants selected from a F_4 progeny are given the F_4 progeny number, *i.e.*, the serial numbers of the F_2 and the F_3 plants from which the progeny was derived, and a new number showing the serial number of the plant among those selected from that F_4 progeny. This system is summarised below.

Generation	*Number*	*Description*
F_3	7911-7	Progeny obtained from plant number 7 selected in F_2.
F_4	7911-7-4	Progeny from plant number 4, selected from the F_3 progeny derived from plant number 7 selected in F_2.
F_5	7911-7-4-2	Progeny from plant number 2, selected from F_4 progeny derived from plant number 4 selected from F_3 progeny obtained from plant number 7 selected in F_2.
F_6	7911-7-4-2-8	Progeny from plant number 8, selected from F_5 progeny dervied from plant number 2 selected from F_4 progeny of plant number 4 selected from F_3 progeny of plant number 7 selected in F_2.

In this system, the pedigree of a progeny is immediately known, and one does not have to refer to the previous years' records. But there is a greater chance of error in this system since more numbers are to be recorded. The breeder may devise his own system of record keeping. *In both the systems, the progenies are assigned a different serial number when they become homozygous and are included in preliminary yield trials. This serial number is given only to those homozygous lines that are included in preliminary yield trials.*

In the pedigree record, a brief note is made about the distinguishing features of each progeny. In keeping a pedigree record, the following should be kept in mind.

1. Only important characteristics should be recorded. If a large number of other characters are noted, the record keeping would become a great burden.
2. Only promising progenies should be included in the record. Poor progenies may be simply marked 'discard'. This would give more time for studying promising progenies.
3. *The pedigree record must be accurate.* Keeping no record is better than keeping an inaccurate record which will create confusion.

The pedigree record is often useful in the elimination of some progenies. In later generations, *e.g.*, F_5, F_6 etc., if some lines

originated from the same F_4 or F_3 progeny and are similar, only one of these lines need be maintained. Further, it may often be possible to obtain a general idea about the inheritance of characters by studying the pedigree record.

APPLICATIONS OF PEDIGREE METHOD

The pedigree method is the most commonly used method for selection from segregating generations of crosses in self-pollinated crops. This method is often used to correct some specific weaknesses of an established variety (*combination breeding*). Pedigree method is also useful in selection of new superior recombinant types. It is almost always hoped that some transgressive segregants would be recovered (*transgressive breeding*). Thus the method is suitable for improving specific characteristics, such as, disease resistance, plant height, maturity time etc., as well as yield and quality characteristics. Even when the objective of the breeder is to correct specific weaknesses of a variety, he generally expects to improve the yield and quality as well.

THE PROCEDURE FOR PEDIGREE METHOD

A general outline of the pedigree method is given below (Fig. 12.1). However, the breeder may modify the procedure according to his specific needs.

Hybridization. The selection of parents to be used in a cross is the most important step in a breeding programme based on hybridization (see, Chapter 6). The selected parents are crossed to produce a simple or a complex cross. For convenience, we shall refer to the seed obtained from both simple and complex crosses as the F_1 seed.

F_1 Generation. F_1 seeds are space-planted so that each F_1 plant produces the maximum F_2 seed. Ordinarily, 15-30 F_1 plants should produce enough seed for a good F_2 population size.

F_2 Generation. In F_2, 2,000-10,000 plants are space-planted to facilitate selection. About 100-500 plants are selected and their seeds are harvested separately.

Usually, the F_2 population size should be 10-100 times the number of F_2 plants that are to be selected, that is, the number of F_3 progenies it is possible to handle. The number of F_3 progenies may vary from 100 to 500 depending upon the facilities available and the objectives of the breeding programme. When closely related varieties are crossed, the number of F_3 progenies maintained would be considerably smaller than when the parent varieties are unrelated by descent. When the objective of breeding is to improve yield, to recover transgressive segregants, or both, a relatively larger number of F_2 plants, that is, F_3 progenies, would be selected.

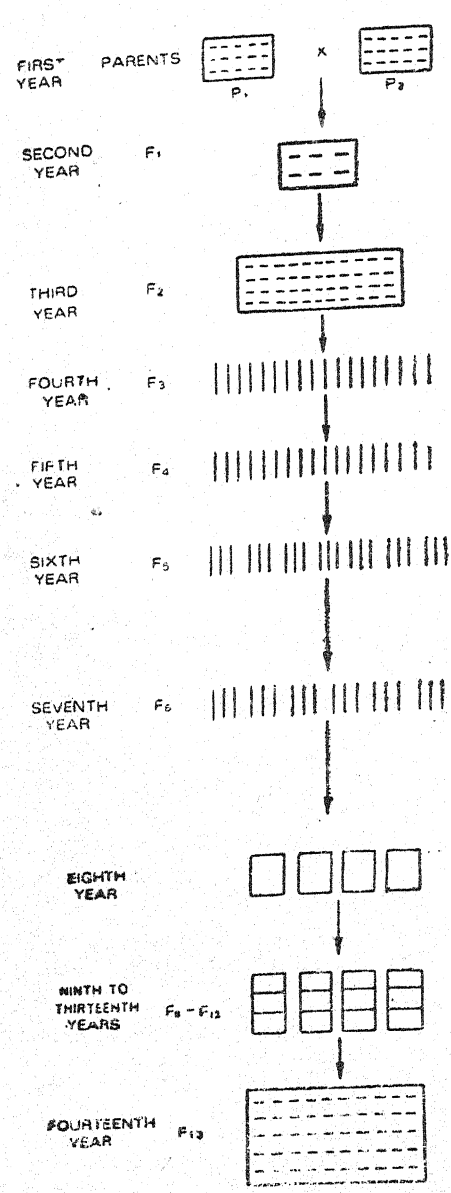


Fig 13.1. A simplified schematic representation of the pedigree method of handling the segregating generations from crosses in self-pollinated crops.

The selection in F_2 is based on characteristics that are simply inherited, *e.g.*, plant height, head type, seed colour, disease resistance, presence of awns etc. Selection for vigour is generally useless because vigour may be due to heterozygosity, environment or G×E interaction. Selection for characters like yield or quality are generally ineffective because these characters are greatly affected by the environment. The value of selection will largely depend upon the skill of breeder. This is because he has to judge which F_2 plant would produce superior progeny for characters like yield ; this he cannot determine on the basis of measurements taken on the F_2 plants (why ?). This is the skill that a breeder develops through a close and deep study of the concerned crop species. It may be emphasized that the breeder *must not select too many F_2 plants. He should select only as many F_2 plants as he can handle efficiently in the subsequent generations with the facilities at his disposal.*

F_3 Generation. Individual plant progenies are space-planted. Each progeny should have about 30 or more plants. Individual plants with desirable characteristics are selected, particularly from superior progenies. Outstanding plants from inferior progenies may also be selected. Disease and lodging susceptible progenies and progenies with undesirable characteristics are eliminated. The number of plants selected in F_3 should be preferably less than the number of F_3 progenies. If the number of superior progenies is small, the whole cross may be rejected.

F_4 Generation. Individual plant progenies are space-planted. Again desirable plants are selected mainly from superior progenies. The number of plants selected in F_4 is generally much lower than the number of F_4 progenies. Progenies with defects and undesirable characteristics are rejected. Progenies are compared visually, and more plants are selected from superior progenies. If two or more progenies coming from the same F_3 progeny are similar and comparable, only one may be saved and the others may be rejected. Thus *the emphasis is on selection of desirable plants from superior progenies.*

F_5 Generation. Individual plant progenies are generally planted according to the recommended commercial seed rate. Often three or more rows are grown for each progeny to facilitate comparison among progenies.

Many families may have become reasonably homozygous and may be harvested in bulk. In families showing segregation, individual plants are selected. The breeder has to visually assess the yielding potential of progenies and reject the inferior ones The number of progenies must be reduced to a size manageable in preliminary yield trials, which is usually 25-100 progenies.

F_6 Generation. Individual plant progenies are planted in multi-row plots and evaluated visually. Progenies are harvested in bulk since they would have become almost homozygous. Progenies showing segregation may be eliminated unless they are outstanding ; in such progenies individual plants may be selected.

Preliminary yield trial may be planted for those progenies which are reasonably homozygous and have enough seed. Inferior progenies are eliminated based on yield data from preliminary yield trial and/or visual evaluation.

F_7 Generation. Preliminary yield trials with 3 or more replications are conducted to identify few superior lines. The progenies are evaluated for plant height, lodging and disease resistance, flowering time, maturity time etc. Quality tests may serve as additional criteria for selection. Standard commercial varieties must be included as checks for comparison. Two to five outstanding lines, if superior to checks, would be advanced to the coordinated yield trials.

F_8 to F_{11} Generations. The superior lines are tested in replicated yield trials at several locations for two (in case of barley) to four (in case of wheat) years. During these trials the lines are evaluated for yield, disease and lodging resistance, maturity time and quality etc. A line that is superior to the best commercial variety in yield and other characteristics would be released as a new variety.

F_{12} Generation. When a strain is likely to be released as a variety, the breeder usually multiplies its seed during its last year in trial. The breeder has the responsibility to supply the breeder's seed to the National Seeds Corporation which is responsible for the production of foundation seed. Thus in F_{10} to F_{13} (depending on the crop), the seed of the new variety will be multiplied for distribution to the farmers.

BASIS OF SELECTION

In the segregating generations, thousands of plants have to be evaluated for the selection of desirable plants. Almost always, it is not possible for the breeder to take measurements on the various characters and base the selection on these measurements. Therefore, the breeder has to base the selection on quick visual evaluation of the plants. Selection for morphological characters like height, leaf size and shape, head type, grain colour, presence of awns etc. and for certain other easily observable characters like days to flowering, days to maturity, disease resistance etc., is easy because these characteristics are easily observed and evaluated visually. Selection for many of these characters during the segregating generations is reasonably effective because they are generally governed by one or a few major genes, and often they have moderate to high heritability.

Although characters like disease resistance are easily observable, the differentiation between resistant and susceptible plants depends upon a uniform attack of the disease on all the plants of a population. Disease spread is rarely uniform even in a completely susceptible population, and the intensity of disease outbreak greatly varies from year to year. Therefore, the breeder may find it necessary to create uniform and heavy disease outbreaks by artificial inoculations,

i.e., artificial epiphytotics (Chapter 20). Similarly, selection for lodging resistance depends upon the environmental conditions that lead to lodging. Such conditions do not occur every year. The breeder, therefore, generally has to select for the breaking strength of straw and for the force required to uproot the plants. These characteristics are positively correlated with lodging resistance. Selection for cold tolerance may have to be based on the ability of plants to tolerate artificial low temperatures. This may be necessary because the natural temperature may not go down to the critical level every year.

Selection for quality characters often requires detailed tests. Each crop will have a different set of quality characteristics depending upon its use. For example, in case of wheat milling, baking and chapati making qualities are important, while in barely malting quality is valuable. Often the basis of quality is complicated and several tests have to be conducted for its proper evaluation. For example, baking quality in wheat is determined by the following tests : percentage of protein and ash, viscosity of flour, mixing time and water absorption by flour, loaf volume etc. In cases where quality tests are complex and time taking, quality tests are generally delayed till F_7 or F_8 when the number of progenies has been reduced to a reasonable size. But quality tests have to be simple, quick and cheap to serve as a useful basis for selection in the segregating generations. Some quality tests are simple, rapid and inexpensive, and are widely used, *e.g.*, determination of brix in sugarcane, of a cyanogenic glucoside in sudan grass etc.

The most discouraging aspect of the breeding methods is that there is no simple, quick and reliable method of selection for yielding ability in the segregating generations, particularly in F_2 to F_4 where single plants are selected. This is primarily because yield has a very low heritability in the early segregating generations, and yield of individual plants is generally unreliable in predicting the yielding ability of their progeny. Space planting, heterozygosity, environmental influences and G×E interactions all contribute to the low heritability of yield. This problem is discussed in more detail later.

EARLY GENERATION TESTS

Some parents combine well to produce superior progeny, while others do not. Similarly, some F_2 plants from a cross would be more likely to produce superior progeny than others. If crosses and plants likely to produce superior progeny are identified at an early stage, say in F_2, F_3 or F_4, much labour, space, time and money would be saved. Thus the objective of early tests is to select (1) superior crosses, and (2) superior plants from within superior crosses.

Selection among Crosses. Performance, that is yield, of F_2, F_3 or F_4 bulks of different crosses gives some information about the performance of the progenies derived from them. This information would be reliable if the yield trials are conducted at more than one

location in two or more years. Poor yielding crosses are generally less likely to produce better yielding progenies than the higher yielding ones. Early generation bulk tests may, therefore, help the breeder in discarding the poor crosses and allow him to give more attention to the superior crosses.

A more reliable information about the potential of crosses may be obtained through F_3 or F_4 progeny trials. A reasonably large number, about 100 or more, of individual plant progenies from each cross are grown in a replicated trial at two or more locations and the trial is perferably conducted for two or more years. From these data, mean yielding ability of the crosses, genotypic variance among the progenies and expected genetic advance under selection may be estimated (Chapters 4--6). A desirable cross should have high mean yield, high genetic variance and high expected genetic advance under selection. It may be stressed that the trial should be conducted over locations and years, wherever possible, for a reliable estimate of the genetic variance. The values obtained from trials at a single location may be misleading. Why ?

Although such detailed trials would require time, space, labour and funds, the elimination of poor crosses may more than compensate for them. This not only saves times and other facilities by reducing the number of progenies to be handled subsequently, but provides the breeder with more time to concentrate on crosses from which he is more likely to select a superior progeny. Even selection based on a single trial at one location may be helpful in discarding the poor crosses. At CIMMYT, Mexico, wheat crosses are regularly evaluated in F_2, and the inferior crosses are rejected. Only superior crosses are taken to F_3 and later generations.

Selection within Crosses. Selection based on characteristics of single plants in F_2 is effective for characters with high heritability, but it is ineffective for those characters that have low heritability, *e.g.*, yield. The efficiency of selection for yield in F_2 may possibly be improved by using some selection schemes. Gardner used a *grid method* for effective selection for yield (3% yield increase/generation for 16 generations) in maize. In this method, the plot in which selection is to be done is divided into a number of small subplots or grids (each having 40 plants or so). Selection is carried out in each subplot separately, and no comparison is made among the subplots. The basis for this method is that the environmental effects within the smaller subplots will be relatively smaller than those in the entire plot. Therefore, division of the plot into grids is expected to reduce the environmental influences and increase the effectiveness of selection. This method has found some application in cross pollinated crops, but has not been tried in self-pollinated species. Another scheme, *honeycomb design*, has been proposed by Fasoulas to overcome the confusing effects of the environment on individual plant yields. In this design, individual plants are planted at a distance sufficient to eliminate all interplant competition, and in a manner so that they form hexagons around every plant in the field. The

plant in the centre of a hexagon is compared with all the plants on the periphery of the hexagon; the central plant is selected only when it outyields all other plants in the hexagon. The hexagon is moved successively so that each plant in the field occupies the central position in one of the hexagons thus formed. The intensity of selection is varied by changing the size of the hexagon, *i.e.*, having one, two or three hexagonal rows of plants in a hexagon to yield 14.3, 5.3, and 2.7 per cent selection intensities, respectively. Thus the honeycomb design uses the principle of moving average, but in the case of single plant data. There are conflicting reports on the effectiveness of this design, and there is no convincing evidence in its favour. In any case, the design (1) requires a special planter designed for the purpose, (2) considerable precaution in planting of the experiments and in recording of data are needed, and (3) much more land is required due to the much larger than the normal spacing among the plants.

The above techniques (1) involve yield measurements on thousands of individual plants which is time consuming and cumbersome, and (2) their effectiveness is not clearly established. A relatively more successful scheme appears to be the indirect selection for yield. This is done by selecting for an easily measurable character which has high heritability and exhibits a high positive correlation with yield. A well known example of such a scheme is the selection for increased ear number/plant (prolificacy) in maize by Lonnquist and others. Ear number/plant in maize has a high positive correlation with yield, is easily observable and measurable, and has high heritability. However, such handy characters are not available in many crops, and it is doubtful if the same trait will be equally useful in every population of a crop species, or through several selection cycles in the same population. Apparently, the breeder has to solely rely on visual selection based on his skill and judgement for the identification of F_2 plants likely to yield superior F_3 progenies since, unfortunately, there does not appear to be a reliable tool to assist him in this matter. A good breeder partly develops this skill through a painstaking and deep study of the crop plant, and partly he has a natural gift for it. A good breeder generally has the feel of the type of progeny a plant is likely to produce. It is, therefore, not surprising that the available evidence indicates that, at least in cereals, visual selection in F_2 is as effective as selection based on yields of individual F_2 plants.

Replicated trials of F_3 or F_4 progenies provide a reasonably reliable basis for selection among progenies for yield and other characteristics with low heritability, particularly when the trials are conducted over locations and years. At the least, such trials should be conducted at more than one location to be of some value in selection. The breeder may, however, reject only the poorest yielding progenies to be on the safe side. There are two main schemes for evaluation of the usually large number of F_3 progenies selected from a cross. These schemes attempt to minimise the effects of soil

heterogeneity and other environmental factors and thereby increase the efficiency of selection.

Contiguous Control. One row or plot of a control variety is planted after every two rows (or plots) of F_3 progenies. This ensures that each F_3 progeny is located next to the control variety. The yields of F_3 progenies are expressed as per cent of that of the control variety; these values are then used for comparison among the progenies to identify the superior F_3 progenies. It is expected that the variation in soil fertility and other environmental factors will be expressed in the yield of the control variety. Therefore, the estimates of yields of the F_3 progenies in relation to the check variety would show considerably reduced variation due to the environmental factors as compared to their actual yields. The check variety may be planted after every four or six (instead of two) F_3 rows to reduce the land requirement. In such a case, the yield of an F_3 progeny is expressed as per cent of that of the nearest check row or plot.

Moving Average. In this method, F_3 progenies are planted in a random order, and no check variety is included in the trial. The yield of an F_3 progeny is expressed as per cent of the average yield of seven F_3 progenies, *i.e.*, the progeny in question and the nearest three progenies on its either side. The yields of the first three and the last three progenies in a block are expressed as per cent of the average yield of the seven nearest progenies, including the progeny in question. One may use the average yield of more or less number of progenies for the comparison. Thus for comparison of each F_3 progeny, a different average is calculated ; this average serves the same purpose as the check variety in the contiguous control method. There is some evidence that the contiguous control and the moving average methods may be of comparable efficiency in the identification of superior yielding F_3 progenies; the latter, however, has the advantage of requiring less land and other resources as plots of a control variety are not planted.

The cost and effort needed for the detailed replicated trials over locations and years may not be justified in view of the limited progress made under selection. It appears that the accepted practice of selection based on easily observable characters and visual evaluation in early generations is a sound one, and no alternative approach of proven effectiveness is available at present. Postponing the yield trials to F_4 to F_8 appears to be justified in view of the low heritability of yield in the early generations.

OFF-SEASON CROPS

The time needed for developing a veriety may be reduced if off-season crops are grown at suitable locations. F_1 generation may be safely advanced to F_2 in greenhouse or in an off-season nursery. Segregating populations may also be advanced in case they are

bulked. The land and other facilities at off-season nurseries or in a greenhouse are generally limited. Therefore, it may not be possible to grow a large number of progenies in them. Off-season nurseries or greenhouses cannot be used for making selections, except for simply inherited characters. This is because the environment in these cases would generally not be comparable to that at the place for where the variety is being developed. An off-season crop of wheat (*T. acstivum*), barley (*H. vulgare*), etc. may be grown at Wellington (T.N.), of rice (*O. sativa*) at Cuttack (Orissa), two off-season crops of maize (*Z. mays*) at Dholi (Bihar) and Varanasi (U.P.), of pulses at Sangla (Kinnaur) and Lahaul-Spiti ((H.P.), and of gram (*C. arietinum*) at Taper-Waripore (J.K.).

MERITS OF PEDIGREE METHOD

1. The method gives the maximum opportunity for the breeder to use his skill and judgement for the selection of plants, particularly in the early segregating generations.
2. It is well suited for the improvement of characters which can be easily identified and are simply inherited.
3. Transgressive segregations for yield and other quantitative characters may be recovered in addition to the improvement in specific characters.
4. It takes less time than the bulk method to develop a new variety.
5. The breeder may often be able to obtain information about the inheritance of qualitative characters from the pedigree record.
6. Plants and progenies with visible defects and weaknesses are eliminated at an early stage in the breeding programme.

DEMERITS OF PEDIGREE METHOD

1. Maintenance of accurate pedigree records takes up valuable time. Sometimes it may be a limiting factor in a large breeding programme.
2. Selection in a large number of progenies in every generation is laborious and time consuming. The breeder would not be able to handle many crosses according to the pedigree method.
3. The success of this method largely depends upon the skill of the breeder. There is no opportunity for natural selection to influence the populations.
4. Selection for yield in F_2 and F_3 is ineffective. If care in not taken to retain a sufficient number of progenies, valuable genotypes may be lost in the early segregating generations.

ACHIEVEMENTS

Once the genetic variability present in *desi* varieties was exploited, hybridization was used for further improvement in the crops. Pedigree method has been the most extensively used method for handling the segregating generations from the crosses. A large number of varieties have been developed by the pedigree method in crops like wheat (*T. aestivum*), rice (*O. sativa*), barley (*H. vulgare*), pulses, oilseeds, cotton (*Gossypium sp.*), tobacco (*Nicotiana sp.*), jowar (*S bicolor*), vegetables etc. Nearly all the improved varieties of self-pollinated crops recommended for cultivation have been developed by the pedigree method. These varieties represent improvements in yielding ability, quality, disease resistance and other agronomic characteristics. A few examples are briefly described below.

Most of the NP wheats (*e.g.*, NP 52, NP 80-5, NP 120, NP 125, 700 and 800 series of NP wheats etc.) were developed by the pedigree method. K 65 wheat is a tall variety recommended for rainfed conditions. It was isolated from the cross C 591 × NP 773. K 68 was developed from the cross NP 773 × K 13 ; it has amber coloured grains and a very high chapati making quality. WL 711 was selected from the cross (S 308 × Chris) × Kalyan Sona. It is a double dwarf variety and is very high yielding, but it is highly susceptible to Karnal bunt. Malviya 12 wheat has amber, hard, medium bold grains and is suitable for early as well as timely sown conditions. It performs well even at low fertility with restricted irrigation. Malviya 12 was developed from the cross NP 876 × Cno 66.

In rice breeding, Taichung Native 1 and IR 8 have contributed directly or indirectly in the development of many high yielding popular varieties. The cross Taichung Native 1 × T 141 produced two outstanding rice varieties Jaya and Padma ; Padma has shorter duration and finer grains than Jaya. Other varieties developed through the pedigree method are Bala, Cauveri, Karuna, Krishna, Ratna, Sabarmati etc.

As noted previously, *G. hirsutum* variety Gadag 1, a pureline selection from Dharwar-American variety, was susceptible to red leaf blight. To remove this defect, Gadag 1 was crossed to Combodia Coimbatore 2 (CC 2) which is resistant to red blight (CC 2 is a pure-line selection from the Combodia cotton of Madras). From the cross Gadag 1 × CC 2, variety Laxmi was selected. Laxmi is superior to Gadag 1 in ginning outturn, fibre properties, earliness and resistance to leaf blight.

Pusa Early Dwarf is a short statured early maturing variety of tomato. It was developed by pedigree selection from the cross Meeruti × Red Cloud. Red Cloud is an early dwarf variety introduced from U.S.A. The fruits of Pusa Early Dwarf are medium sized and slightly flattish. It gives about 33 per cent more yield in the first pick and about 25 per cent more total fruit yield than Pusa Ruby.

SUMMARY

In pedigree method, individual plants are selected till the progenies become homozygous. In F_5 and onward, selection within families becomes less and less effective due to increasing homozygosity. A record of all parent-offspring relationships is maintained so that each paogeny can be traced back to the F_2 plant from which it originated. Selection for easily identifiable characters with high heritability is effective in F_2 and subsequent generations. But selection for complex characters like yield that have low heritability is ineffective. Preliminary yield trial is generally conducted in F_6 or F_7. Replicated yield tests are conducted for upto four years at several locations. The best line is then released as a new variety.

Early generation tests may be used to identify superior crosses. But identification of superior plants within crosses in F_2 or F_3 is generally not feasible due to high effects of the environment on yield. Special techniques are available to assist in selection for disease resistance, lodging resistance, quality etc. Off-season nurseries may be used to advance a generation, *e.g.*, from F_1 to F_2, which would save the time required for developing a new variety.

The pedigree method provides the best opportunity for the breeder tc exercise his skill in selection. It also provides opportunity for selection of transgressive segregants. But the method requires considerable attention from the breeder. This limits the number of populations he can handle. Keeping of accurate pedigree records is time consuming, and often it is a burden.

QUESTIONS

1. Write short notes on the following : (i) pedigree record, (ii) early generation tests, and (iii) off-season nurseries.
2. Briefly describe the procedure of pedigree method of breeding. Discuss the achievements through this method of breeding.
3. Describe the basis of selection in the segregating generations. Discuss the merits, demerits and applications of pedigree method.

Suggested Further Readings

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CHAPTER 14

Bulk Method

The bulk method of breeding was first used by Nilsson-Ehle in 1908 at Svalof. This method is also known as the *mass method* or the *population method* of breeding. *In the bulk method, F_2 and subsequent generations are harvested in mass or as bulks to raise the next generation. At the end of the bulking period, individual plants are selected and evaluated in a similar manner as in the pedigree method of breeding.* The duration of bulking may vary from 6-7 to 30 or more generations. During the bulking period, artificial selection may or may not be practised. The essential difference between the bulk and the pedigree methods, therefore, lies in the manner in which the segregating generations are handled ; in the pedigree method, individual plant progenies are grown and evaluated in F_3 and the subsequent generations, while in the bulk method these generations are grown as bulks.

APPLICATIONS

The bulk method is suitable for handling the segregating generations of cereals, smaller millets, most of grain legumes and oilseeds. It may be used for three different purposes : (1) isolation of homozygous lines, (2) waiting for the opportunity for selection, and (3) to provide opportunity for natural selection to change the composition of the population.

Isolation of Homozygous lines. The most common use of the bulk method is for the isolation of homozygous lines with a minimum of effort, and expense. The population is carried to F_6 or F_7 as a bulk ; by this time the population approaches homozygosity. Individual plants are selected and evaluated as in the pedigree method. Since the selected plants would be almost homozygous, most of the progenies will be homogeneous. Therefore, a preliminary yield trial may be planted in the second year after selection of the individual plants.

Waiting for the Opportunity for Selection. Selection for resistance to diseases, lodging, cold etc. depends upon the presence of suitable environmental conditions favouring disease epidemic, severe lodging, cold-killing etc. Such environments do not occur every year. The segregating generations may be carried in bulk until such environments occur. Individual plants are then selected and handled as in the pedigree method. The duration of bulking in this case would depend upon the occurrence of the particular environment ; it may end in F_2 itself or may continue upto F_6 or beyond. This method is generally known as the *Mass-Pedigree Method of Harlan.*

Opportunity for Natural Selection. Maintenance of bulks is inexpensive and without much effort. Some bulk populations may be carried upto F_{20} or F_{30} to provide an opportunity for natural selection to act. In such a case, the bulking period has to be fairly long because upto F_6 the population would be, to some extent, heterozygous and natural selection would act on heterozygous plants. But after F_7, natural selection would act on homozygous plants and would change the frequency of homozygous genotypes present in the population. It is assumed that natural selection would favour higher yielding genotypes and eliminate the poorer genotypes. Therefore, from a population maintained as a bulk for a long period, one may expect to isolate superior lines at a much higher frequency than from the F_2 of the same cross. This method was termed as the *Evolutionary Method of Breeding* by Suneson as it allows natural selection to act on the population and change its genotypic composition.

THE PROCEDURE FOR BULK METHOD

The exact procedure for the bulk method would vary depending upon the objective of breeder. The following procedure is described for the isolation of homozygous lines (Fig. 14.1). The breeder may introduce various modifications in the scheme to suit his needs.

Hybridization. Parents are selected according to the objective of the breeding programme. A simple or a complex cross is then made depending upon the number of parents involved.

F_1 Generation. F_1 is space-planted and harvested in bulk. The number of F_1 plants should be as large as possible ; usually more than 20 plants should be grown.

F_2-F_6 Generation. F_2 to F_6 generations are planted at commercial seed rates and spacings. These generations are harvested in bulk. During this period, environmental factors and disease and pest outbreaks would change, to some extent, the frequencies of the different genotypes in the population. Artificial selection is generally not done. The population size should be as large as possible, preferably 30,000-50,000 plants in each generation.

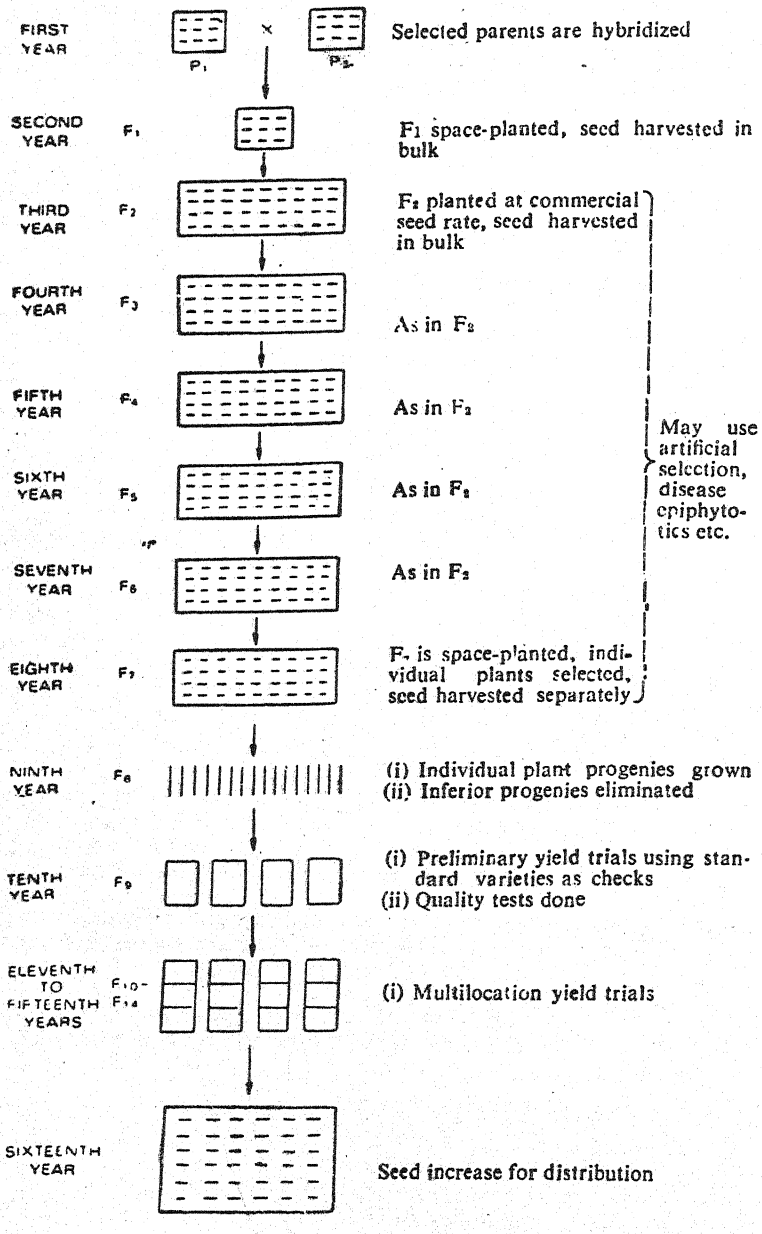


Fig. 14.1. A generalised scheme of bulk method for the isolation of homozygous lines of self-pollinated crops.

F_7 Generation. About 30-50 thousand plants are space-planted. 1000 to 5000 plants with superior phenotypes are selected and their seed harvested separately. Selection is based on the phenotype of plants, grain characteristics, disease reaction, etc.

F_8 Generation. Individual plant progenies are grown in single or multi-row plots. Most of the progenies would be reasonably homozygous and are harvested in bulk. Weak and inferior progenies are rejected on the basis of visual evaluation. Only 100-300 plant progenies with desirable characteristics are saved.

Some progenies would show segregation. Such progenies are generally rejected unless they are of great promise. In promising progenies, individual plants may be selected ; preliminary yield trial will be delayed for one year in such cases.

F_9 Generation. Preliminary yield trial is conducted ; standard commercial varieties are used as checks. The yield is used as a basis for selection of superior progenies. Quality test may be conducted to further reject undesirable progenies. The progenies are evaluated for height, lodging resistance, maturity date, disease resistance and other important characteristics of the crop species.

F_{10}-F_{13} Generations. Replicated yield trials are conducted over several locations using standard commercial varieties as checks. The lines are evaluated for important characteristics in addition to yield, disease resistance and quality. If a line is superior to the standard varieties in yield trials, it would be released as a new variety.

F_{14} Generation. The seed of the released variety is increased for distribution to the cultivators.

ARTIFICIAL SELECTION DURING THE BULKING PERIOD

Generally artificial selection is not done during the bulking period. But the breeder may eliminate the inferior and undesirable types and thus assist natural selection. In long-term bulks of 20-30 generations, natural selection plays an important role, but in the short-term bulks of 6-10 generations the effect of natural selection is likely to be small. Therefore, artificial selection may be desirable in short-term bulks.

Even in long-term bulks, certain desirable characteristics must be selected for because they may not have any selective advantage or may even have a selective disadvantage. For example, seed colour, presence of awns, seed size etc. may not have a selective advantage over the undesirable types. Natural selection would favour the genotypes that produce a large number of seeds irrespective of seed size. Therefore, selection for larger seeds would be desirable. Certain desirable characteristics have a poor survival value in mixed populations. Artificial selection must be practised for such

characteristics to eliminate the undesirable types. A classical example of this type is the dwarf plant type in rice. The dwarf types are poor competitors against the tall, leafy types in a mixed stand. Therefore, tall types must be eliminated from the population to maintain the dwarf characteristics in the population. A similar situation is encountered in wheat as well. In any case, artificial selection would help the natural selection in the elimination of weaker, undesirable and inferior types from the population.

Artificial disease epiphytotics may be created to favour disease resistant types. Insect infestation may be created for selection of insect resistance. Similarly, other techniques may be used to favour various desirable types. For example, earliness may be favoured by harvesting the crop when only a portion of the crop is mature. Larger seed size may be favoured by rejecting seeds smaller than a particular size ; the smaller seeds may be easily separated by passing the seeds over sieves of an appropriate size.

DURATION OF BULKING

The duration of bulking would depend upon the objective of bulking. If the objective is isolation of homozygous lines, 6-8 generations of bulking is enough. In case bulking is done to carry the generation till the suitable environment for effective selection occurs, bulking ends after the occurrence of the critical environment. But if the objective is to allow natural selection to act on the population, long-term bulks of 20-30 generations are desirable. The population would have partly heterozygous plants till F_6 or F_7 and only by F_{10} the population may be regarded as a mixture of homozygous genotypes. Natural selection on heterozygous plants will be of little importance because such genotypes would segregate to produce other genotypes. The natural selection becomes important only when the population has beeome homozygous. Therefore, for natural selection to exert some effect on the frequency of genotypes, the bulking should be continued for some generations beyond F_{10} preferably to F_{20} or F_{30}.

NATURAL SELECTION DURING BULKING

The survival of a genotype in a mixture depends upon the number (but not weight) of seeds produced, and the percentage of plants produced by these seeds that reach maturity to produce progeny. Theoretically, the best genotype would increase rapidly in a mixture, while the poorest types would be eliminated. The medium or average types would show an initial increase, followed by a decrease when the poorest types would have disappeared. The second best types would begin to decrease only when the poor types are almost eliminated from the population. Ultimately, only the best type would remain, but the second best types would be slow to disappear. This theoretical consideration is complicated by two

factors : *first,* the seed production rate of the best type may not be the same when competing against the poorest, the average and the second best types ; and *second,* the environment in different years may favour different types in place of favouring only one type in all the years.

Natural selection exerts considerable influence on the composition of populations. Evidence favouring this conclusion comes from studies on the survival of genotypes in mixtures and in hybrid populations. Studies with *varietal mixtures* show that one or two varieties become dominant, while the others decrease in frequency. But at some locations, the change may be slow and relatively more varieties may be maintained in the mixture. Different varieties may become dominant at different locations. Generally, the dominant varieties would be agronomically superior. More importantly, inferior types are regularly eliminated. But often the ability of survival in competition may not be related to agronomic superiority. Agronomically desirable characteristics like date of flowering, date of maturity, plant height, disease resistance, determinate habit of growth etc. may not be related to competitive ability. On the other hand, such characters may be selected against by natural selection, *e.g.*, dwarf plant types in rice. However, it is obvious that natural selection is effective in changing the genetic composition of populations.

Survival in hybrid populations has been measured in terms of (1) changes in the frequency of specific genes, (2) performance of hybrid populations, and (3) frequency of superior progenies isolated from hybrid populations, *Survival of genes* in hybrid populations has been studied in detail by Suneson and coworkers in the barley composite cross II. The composite cross II (note that not a complex cross) is a mixture of equal amounts of seed from 377 F_1s produced from 218 varieties crossed in all possible combinations, except one. It was found that agronomically inferior genes, *e.g.*, hooded, deficient, black lemma, rough awn, are quickly eliminated from the population. The degree and, in some cases, the direction of change (*e.g.*, two-row versus six-row) was greatly affected by the location. Some seemingly neutral genes may be eliminated possibly due to their linkage with inferior genes.

The *yield* of composite cross II was studied from F_8 to F_{24} and compared with the standard variety Atlas. The yield of composite cross II was initially only 67 per cent of that of Atlas. But it increased gradually, and in F_{24} the composite cross II out-yielded Atlas by about 36 per cent. Other evidences also suggest that the performance of bulk hybrid populations improves with the period of bulking. Thus natural selection tends to increase the frequency of higher yielding and agronomically superior genotypes in the bulks. This point was demonstrated by Suneson by isolating and evaluating lines from the Composite Cross II at different stages during the bulking period. More superior progenies were isolated from later generations than from earlier generations. For example, out of the

536 selections in F_{12}, none was of good agronomic type as well as superior in yield to Atlas. In F_{20}, two of the 50 progenies isolated were outstanding agronomically and outyielded Atlas by 37 per cent. And in F_{24}, each one of the 66 selections was superior to Atlas in yield and of moderate to good agronomic type. Three top-yielding progenies outyielded Atlas by 56 per cent. Many plant breeders are skeptical of these findings, which is evident from the lack of a widespread application of the bulk method. It is only natural to expect that a breeding method promising an increase of 56% over the best commercial variety, and requiring relatively small effort should have been extensively used.

It may be concluded that natural selection affects considerably the frequency of genotypes and genes both in varietal mixtures and in hybrid populations. Natural selection may not always favour the agronomically desirable types. But the available evidence indicates that the yield of bulks increases with time, and later generation bulks yield more number of superior progenies than those in earlier generations. Thus bulks method of breeding may be helpful in developing populations that are more likely to yield superior progenies. In view of this, the breeder may carry some bulk populations for this purpose in addition to his other breeding programmes. It may be pointed out that this would not involve much commitment of funds, labour and time of the breeder.

A MODIFICATION OF THE BULK METHOD

A modification of the bulk method based on artificial selection in F_2 and the subsequent generations is outlined below.

F_2 and F_3 Generations. F_2 and F_3 generations are space-planted and a large number (1,000-5,000) of desirable plants are selected. The seeds from selected plants are bulked.

F_4 Generation. F_4 is space-planted and 1,000 to 5,000 desirable plants are selected. Seed from the selected plants is harvested separately.

F_5 Generation. Individual plant progenies are grown. Selection among progenies is based on plant height, disease resistance, lodging resistance, maturity date and other agronomic characteristics. Undesirable and inferior progenies are eliminated. Often only 10-30 per cent of the progenies may be saved. Seed from each of the selected progenies is harvested in bulk.

F_6 Generation. A preliminary yield trial is planted using the bulk seed from the selected individual plant progenies. Observations are recorded on agronomic characteristics and yield. Quality tests may be done on superior progenies.

F_7 Generation. Superior progenies selected on the basis of the yield trial are space-planted for further purification. Individual plants are

selected from the superior progenies, and their seed is harvested separately.

F_8 Generation. Individual plant progenies are grown. Inferior progenies and progenies showing segregation are ordinarily rejected. Each selected progeny is harveged in bulk.

F_9 Generation. Preliminary yield trial is conducted to identify few superior progenies for detailed yield tests. Quality test is done to eliminate undesirable progenies.

F_{10}-F_{13} Generations. Replicated yield trials are conducted at several locations with standard varieties as checks. The line or lines that are superior to the standard checks would be released as new varieties.

F_{15} Generations. Seed of the newly released variety is multiplied for distribution among the farmers.

This modification of the bulk method provides an opportunity for the breeder to exercise his skill and judgement in selection of superior plant types in the early generations (F_2 to F_4). At the same time it does not involve laborious record keeping as in the case of the pedigree method. Therefore, this modification has the appeal that it provides for selection in the early segregating generations and yet it involves less time and labour than the predigree method.

SINGLE-SEED-DESCENT METHOD

Another modification of the bulk method is the single-seed-descent method, which is becoming increasingly popular. In this method, a single seed from each of the one to two thousand F_2 plants is bulked to raise the F_3 generation. Similarly, in F_3 and the subsequent generations one random seed is selected from every plant present in the population and planted in bulk to raise the next generation. This procedure is followed till F_5 or F_6 when the plants would have become nearly homozygous. In F_5 or F_6, a large number (1 to 5 hundred) of individual plants are selected and individual plant progenies are grown in the next generation. Selection is done mainly among the progenies, and the number of progenies is sufficiently reduced to permit replicated trial in the next generation. Individual plants may be selected only from outstanding families showing segregation. Thus preliminary yield trials and quality tests begin in F_7 or F_8 and coordinated yield trials in F_8 or F_9 (Fig. 13.2).

The objective of single-seed-descent method is to rapidly advance the generations of crosses ; at the end of the scheme, a random sample of homozygous or near homozygous genotypes/lines is obtained. F_2 and subsequent generations are grown at very high plant densities as vigour of individual plants is not important. In each year, 2-3 generations may be raised using off-season nurseries

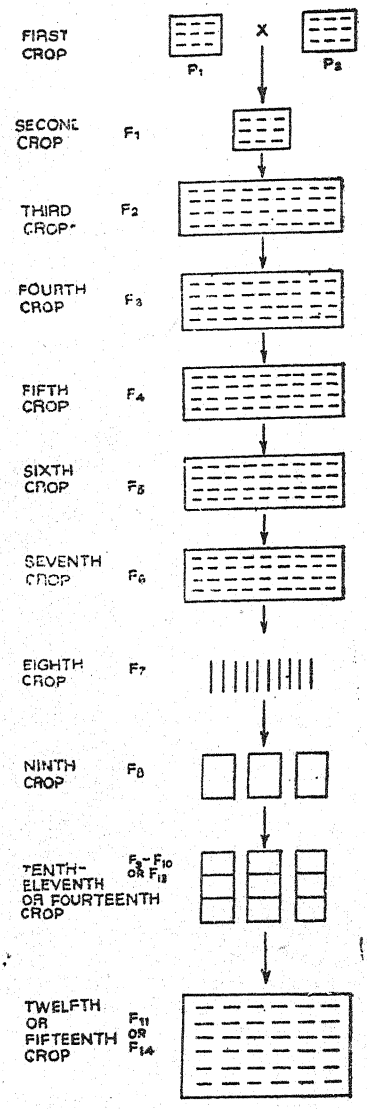


Fig. 14.2. Schematic representation of single-seed-descent method.

Note : Since more than one generation may be grown in each year, the number of generations does not necessarily represent the number of years.

and greenhouse facilities. The important features of this scheme are : (1) lack of selection, natural or artificial, till F_5 or F_6 when the population is reasonably homozygous, and (2) raising of F_3 and later generations from a bulk of one seed from each F_2 and subsequent generation plant in order to ensure that each F_2 plant is represented equally in the end population. As a result of the speed and economy, the single-seed-descent scheme is becoming increasingly popular with the breeders.

The single-seed-descent scheme (1) advances the generation with the maximum possible speed in a conventional breeding method ; (2) requires very little space, effort and labour ; (3) makes the best use of greenhouse and off-season nursery facilities ; and (4) ensures that the plants retained in the end population are a random sample from the F_2 population. However, (1) it does not permit any form of selection (which is implied in the scheme) during the segregating generations ; and (2) in each successive generation, the population size becomes progressively smaller due to poor germination and death of plants due to diseases, insect pests and accidents. In some crops, *e.g.*, pulses, plant loss may be one of the most serious problems of the scheme.

MERITS OF BULK METHOD

1. The bulk method is simple, convenient and inexpensive.
2. Artificial or natural disease epiphytotics, winter killing etc. eliminate undesirable types and increase the frequency of desirable types. The Isolation of desirable types thus becomes much easier.
3. Natural selection increases the frequency of superior types in the population. Progenies selected from long-term bulks are likely to be far superior to those selected from F_2 or short-term bulks.
4. Little work and attention is needed in F_2 and the subsequent generations. The breeder is free to concentrate more on other breeding projects.
5. No pedigree record is to be kept, which saves time and labour.
6. Since large populations are grown, transgressive segregants are more likely to appear and increase due to natural selection. Thus there is a greater chance of isolation of transgressive segregants than in the pedigree method.
7. Artificial selection may be practised to increase the frequency of desirable types.
8. It is suitable for studies on the survival of genes and genotypes in populations.

DEMERITS OF BULK METHOD

1. The major disadvantage of bulk method is that it takes a much longer time to develop a new variety. Natural selection

becomes important only after F_9 or F_{10}, and bulking may have to be done upto F_{20} or more. Thus the time requried is considerably longer, and most breeders do not use the bulk method simply for this reason.

2. In short-term bulks, natural selection has little effect on the genetic composition of populations. But short-term bulks are useful for the isolation of homozygous lines and for specific objectives as in Harlan's Mass-Pedigree Method.
3. It provides little opportunity for the breeder to exercise his skill or judgement in selection. But in the modified bulk method, the breeder has ample opportunity for practising selection in the early segregating generations.
4. A large number of progenies have to be selected at the end of the bulking period.
5. Information on the inheritance of characters cannot be obtained which is often available from the pedigree method.
6. In some cases, at least, natural selection may act against the agronomically desirable types.

ACHIEVEMENTS

In spite of its merits, bulk method of breeding has not been widely used in crop improvement. The possible reasons for the lack of popularity of the bulk method are : (1) the long time required for natural selection to act (more than 10 generations) and, therefore, for developing a variety and (2) the lack of opportunity for the breeder to exercise his skill in the selection of superior plant types during the segregating generations. But the method has been used for studying the survival of genes and genotypes in segregating populations ; the Composite Cross II in barley is the best known example.

The method has been used to a limited extent in barley breeding in U.S.A., and some varieties have originated from bulk populations. Barley varieties Arivat, Beecher, Glacier and Gem originated from a bulk population derived from the cross Atlas × Vaughn ; the bulk was maintained for 7 generations. More than 50 varieties have been developed from composite crosses in barley ; these varieties were developed either directly from the composite crosses or lines isolated from the composite crosses served as their parents.

COMPARISON BETWEEN BULK AND PEDIGREE METHODS

The bulk and pedigree methods differ in the method of handling the segregating generations and in the importance of natural and artificial selections. The end products from both the methods are pureline varieties. The differences between the two methods of breeding are listed in Table 14.1.

Table 14.1. Comparison between bulk and pedigree methods.

	Pedigree method	*Bulk method*
1.	Individual plants are selected in F_2 and the subsequent generations and individual plant progenies are grown.	F_2 and the subsequent generations are maintained as bulks.
2.	Artificial selection, artificial disease epidemics etc. are an integral part of the method.	Artificial selection, artificial disease epiphytotics etc. may be used to assist natural selection. In certain cases, artificial selection may be essential.
3.	Natural selection does not play any role in the method.	Natural selection determines the composition of the populations at the end of the bulking period.
4.	Pedigree records have to be maintained which is often time consuming and laborious.	No pedigree records are maintained.
5.	It generally takes 14-15 years to develop a new variety and to release it for cultivation.	It takes much longer for the development and release of a variety. The bulk population has to be maintained for more than 10 years for natural selection to act.
6.	Most widely used breeding method.	Used only to a limited extent.
7.	It demands close attention from the breeder from F_2 onward as individual plant selections have to be made and pedigree records have to be maintained.	It is simple, convenient and inexpensive and does not require much attention from the breeder during the period of bulking.
8.	The segregating generations are space-planted to permit individual plant selection.	The bulk populations are generally planted at commercial planting rates.
9.	The size of population is usually smaller than that in the case of bulk method.	Large populations are grown. This and natural selection are expected to increase the chances of the recovery of transgressive segregants.

SUMMARY

In the bulk method of breeding, F_2 and the subsequent generations are harvested in bulk with or without artificial selection. At the end of the bulking period, individual plants are selected and their progenies are evaluated as in the pedigree method. The duration of bulking varies with the objective of the breeder. Short-term bulks are useful in isolation of homozygous lines and in exposing the population to specific environments for effective selection of desirable plant types. However, natural selection has little importance in short-term bulks. In long-term bulks, natural selection changes the frequency of different genotypes considerably. In general, inferior and weak genotypes are eliminated by natural selection. But often a desirable characteristic may be selected against by natural selection. Long-term bulks are likely to produce more number of superior progenies than short-term bulks. Artificial selection may be used to assist natural selection.

Bulk method is simple, convenient and inexpensive. During early segregating generations, very little work and attention is needed which gives the

breeder more time to concentrate on other breeding projects. The major disadvantage of the bulk method is that it takes much longer to develop a new variety than the pedigree method. Further, the breeder is unable to exercise his skill and judgement in selection, and therefore the method is less satisfying to him. As a result, bulk method has been only occasionally used; the pedigree method being the most commonly used. The bulk method is suitable for creals, smaller millets, grain legumes and oilseeds.

QUESTIONS

1. Briefly describe the bulk method of breeding and its applications. Discuss its merits and demerits.
2. Discuss the role of artificial and natural selection in the bulk method of breeding.
3. Write short notes on the following : (i) duration of bulking, (ii) survival of genes and genotypes in bulks, (iii) artificial selection in the bulk method, (iv) natural selection in short-term bulks, and (v) achievements of the bulk method.

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CHAPTER 15

Backcross Method

A cross between a hybrid (F_1 or a segregating generation) and one of its parents is known as *backcross*. *In the backcross method, the hybrid and the progenies in the subsequent generations are repeatedly backcrossed to one of the parents.* As a result, the genotype of backcross progeny becomes increasingly similar to that of the parent to which it is backcrossed. At the end of 6-8 backcrosses, the progeny would be almost identical with the parent used for backcrossing. The objective of the backcross method is to improve one or two specific defects of a high yielding variety, which is well adapted to the area and has other desirable characteristics. The characters lacking in this variety are transferred to it from a donor parent without changing its genotype, except for the genes being transferred. Thus the end result of a backcross programme is a well adapted variety with one or two improved characters.

For example, a wheat variety, *e.g.*, Malviya 12, may become susceptible to a disease, *e.g.*, leaf rust, but is an otherwise highly desirable variety. This variety, known as the *recipient parent*, is crossed to a leaf rust resistant variety, the *donor parent*, *e.g.*, Sparrow. The F_1 hybrid and the progeny in the subsequent generations are backcrossed to the recipient parent, *i.e.*, Malviya 12. During the backcross programme, rust resistance is maintained by selection. At the end, rust resistant plants from the progeny are self-pollinated and homozygous rust resistant progenies are isolated. These progenies will be almost the same as the recipient parent, *i.e.*, Malviya 12, except for their rust resistance (as compared to susceptibility to rust of the recipient variety). Since the recipient parent is repeatedly used in the backcross programme, it is also known as the *recurrent* parent. The donor parent, on the other hand, is known as the *non-recurrent parent* because it is used only once in the breeding programme (for producing the F_1 hybrid).

REQURIREMENTS OF A BACKCROSS PROGRAMME

For the successful development of a new variety through backcross method, the following requirements must be fulfilled.

1. A suitable recurrent parent must be available, which lacks in one or two characteristics.
2. A suitable donor parent must be available. The donor parent should have the character or the characters to be transferred in a highly intense form.
3. The character to be transferred must have high heritability. Preferably, it should be determined by one or few genes.
4. A sufficient number of backcrosses should be made so that the genotype of the recurrent parent is recovered in full. Ordinarily, 6-7 backcrosses are sufficient for the purpose.

APPLICATIONS OF BACKCROSS METHOD

The backcross method has been commonly used for the transfer of disease resistance from one variety to another. But it is also suitable for the transfer of other characteristics, even quantitative characters, and is applied to both self- and cross-Pollinated crops. Backcross method may also be modified to allow the recovery of transgressive segregants.

Intervarietal Transfer of Simply Inherited Characters. Characters governed by one or two major genes, *e.g.*, disease resistance, seed colour, plant height etc., are the most suited for transfer through backcross method from one variety of the same species to another. Disease resistance has been the most commonly transferred character. Mostly such transfers are successful, but sometimes they may fail due to a tight linkage between the gene being transferred and some other undesirable gene.

Intervarietal Transfer of Quantitative Characters. Quantitative characters may also be transferred from one variety to another if they have high heritability. The following quantitative characters have been transferred : earliness, plant height, seed size and shape.

Interspecific Transfer of Simply Inherited Characters. Backcross method has been used to transfer simply inherited characters, mostly disease resistance, from related species to a cultivated species. For example, transfer of resistance to wild fire-black fire from *Nicotiana longiflora* to *N. tabacum*, of leaf and stem rust resistance from *Triticum timopheevii*, *T. monococcum*, *Aegilops speltoides* and rye (*S. cereale*) to *T. aestivum*, of black-arm resistance from several *Gossypium* species to *G. hirsutum* etc. Interspecific transfer of genes is easy when the chromosomes of the two species pair regularly. But often chromosomes of different species are differentiated by structural changes that reduce pairing between them. As a result, in interspecific gene transfers often undesirable linked genes are also transferred alongwith the desirable gene. Another difficulty in interspecific transfers is that the gene may not be able to function in the

same way in the genetic environment of the new species (Chapter 25).

Transfer of Cytoplasm. Backcross is used to transfer cytoplasm from one variety or species to another. The transfer of cytoplasm is particularly desirable in cases of cytoplasmic male sterility. The variety or species from which the cytoplasm is to be transferred is used as the female parent. The recurrent parent, the parent to which the cytoplasm is to be transferred, is used as the male parent in the original cross and in the backcrosses. After 6-8 backcrosses, the progeny would have the nuclear genotype of the recurrent parent and the cytoplasm from the donor parent. For example, transfer of *T. timopheevii* cytoplasm to *T. aestivum* has resulted in male sterlie lines of *T. aestivum.*

Transgressive Segregation. Backcross method may be modified to produce transgressive segregants. *Firstly,* the F_1 may be backcrossed only 1-2 times to the recurrent parent leaving much heterozygosity for transgressive segregants to appear. In the *second* modification, two or more recurrent parents may be used in the backcross programme to accumulate genes from them into the backcross progeny. Such a modification of the backcross would produce a new variety that would not be exactly like any one of the recurrent parents.

Production of Isogenic Lines. Isogenic lines are identical in their genotype, except for one gene. Such lines are useful in studying the effects of individual genes (and genes tightly linked to them) on yield and other characteristics. Isogenic lines are easily produced by the backcross method (Chapter 4).

Germplasm Conversion. In some crops, valuable germplasm can not be utilized in breeding programmes since these lines are photosensitive and, as a result, do not flower under the prevailing photoperiods. Such lines may be used as recurrent parents in separate backcross programmes with a photoinsensitive non-recurrent parents for the transfer of photoinsensitivity. The lines derived from these programmes would be photoinsensitive forms of their recurrent parents. These new lines are called *converted lines* and the process is commonly known as *germplasm conversion.* Germplasm conversion makes possible the exploitation of such germplasm which would otherwise be unavailable to the breeder.

For example, sorghum land-races from Africa are disease resistant and possess excellent grain quality, but are photosensitive. Consequently, they fail to flower in India. Some of these land-races have been converted into photoinsensitive lines at ICRISAT, Hyderabad; these converted lines can now be utilized in different breeding programmes.

GENETIC CONSEQUENCES OF REPEATED BACKCROSSING

The genetic consequences of repeated backcrossing may be summarised as follows.

1. Repeated backcrossing leads to homozygosity at the same rate as selfing. Consider a single gene *Aa*. Selfing the F_1 (*Aa*) would produce $\frac{1}{4}$ *A A*, $\frac{1}{2}$ *A a* and $\frac{1}{4}$ *a a*, *i.e.* $\frac{1}{2}$ heterozygotes and $\frac{1}{2}$ homozygotes. Backcrossing the F_1 to the parent *AA* would produce $\frac{1}{2}$ *A A* and $\frac{1}{2}$ *A a*, that is, $\frac{1}{2}$ homozygotes and $\frac{1}{2}$ heterozygotes. In the subsequent backcross generations, the proportion of heterozygotes would decrease to 50 per cent of that in the previous generation (Fig. 15.1), which is the same as in the case of selfing. When several genes are considered together, the proportion of homozygotes for all the genes in a backcross generation is given by the same formula, $[2^m-1)/2^m]^n$, applicable to selfing. *Thus the first effect of repeated backcrossing is a rapid increase in homozygosity and in the frequency of homozygotes.*

2. In a backcross progeny ($\frac{1}{2}$ *A A*, $\frac{1}{2}$ *A a*), the frequency of homozygotes for the allele from the recurrent parent is $\frac{1}{2}$, while in F_2 ($\frac{1}{4}$ *A A*, $\frac{1}{2}$ *Aa*, $\frac{1}{4}$ *aa*) it is only $\frac{1}{4}$. As the number of genes that are segregating increases, the frequency of the desirable homozygotes, *i.e.*, of the recurrent parent type, decreases much more rapidly in the case of selfing than in the case of backcross. For *n* number of genes, this frequency is $(\frac{1}{2})^n$ in the case of backcross progeny, but only $(\frac{1}{4})^n$ in the selfed progeny. Thus it is easier to select for a desirable genotype from a backcross progeny than from a selfed progeny.

When backcrossing is repeated, the frequency of desirable homozygote increases rapidly (Fig. 15.1) and in the sixth backcross progeny, more than 98 per cent of the plants have the *AA* genotype. The same is true for the other genes of the recurrent parent. Therefore, the genes from the nonrecurrent parent are rapidly replaced by those from the recurrent parent. As a result, *the genotype of the backcross progeny becomes increasingly similar to that of the recurrent parent. The rate of this replacement is the same as the rate of increase in the frequency of the AA genotype.* Consequently, after 7 backcrosses more than 99 per cent of the genes of backcross progeny would be from the recurrent parent (Table 15.1). This is expected when no selection is done in the backcross progenies. But often selection would be done for the plant type of the recurrent parent. This will result in a faster rate of return of the backcross progenies to the genotype of the recurrent parent.

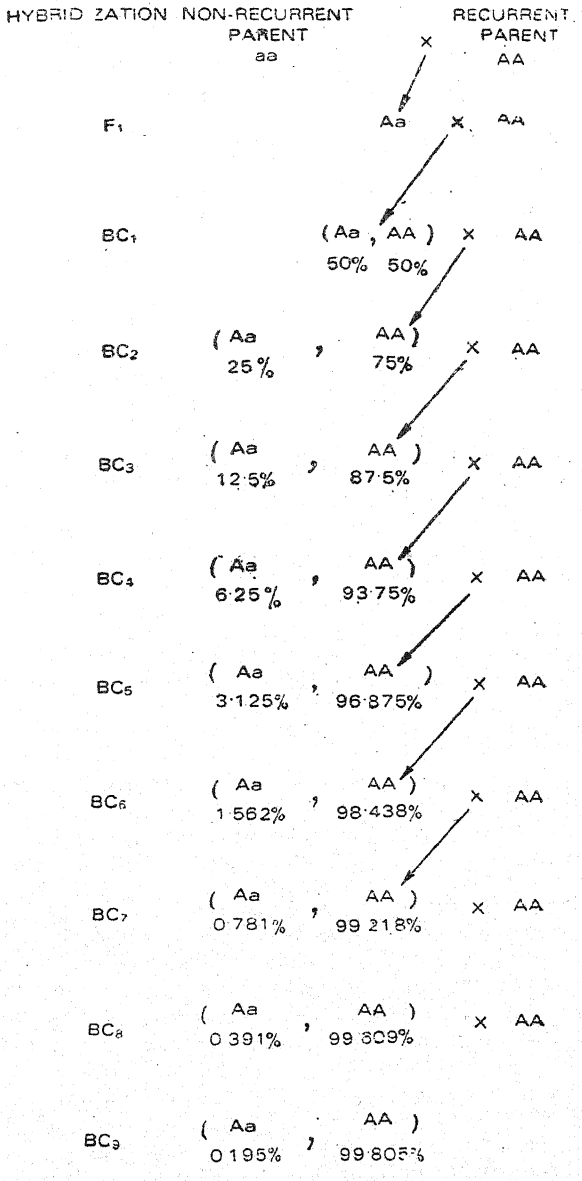


Fig. 15.1. Effect of repeated backcrossing on the frequency of homozygotes and heterozygotes at a single locus *Aa*. (The proportion of *AA* is also the proportion of genes from the recurrent parent).

Table 15.1. Average proportion of genes from the recurrent parent in different generations in a backcross programme.

Generation	*Average proportion (in per cent) of genes from the recurrent parent*
F_1	50
BC_1	75
BC_2	87.5
BC_3	93.75
BC_4	96.875
BC_5	98.438
BC_6	99.218
BC_7	99.609
BC_8	99.805
BC_9	99.902
BC_{10}	99.951

3. *The gene under transfer must be maintained by selection in the backcross generations. Otherwise it will also be replaced by its allele from the recurrent parent.* This gene and the genes tightly linked to it would thus remain in heterozygous state in the backcross generations. Therefore, *there would be opportunity in each backcross generation for crossing over to occur between the gene being transferred and the genes tightly linked with it.* However, when genes are tightly linked (crossing over 0.1 per cent or less), large backcross populations would have to be grown to obtain recombinant types. Often tightly linked genes may remain together upto 10 or more backcross generations.

SELECTION OF PARENTS

It is clear that the backcross method of breeding changes the genotype of the recurrent parent only for the gene under transfer. That is, only the defect of the recurrent parent is removed through backcross method. But some unexpected changes in one or more characters may occur due to genes tightly linked with the gene or genes being transferred. *Therefore, the recurrent parent must be the most popular variety of the area, which has high yielding ability, desirable quality and high adaptability.* In each crop, one or two varieties dominate, and they are very popular with the cultivators. Such a variety may have one or two defects. For example, it may be susceptible to a disease or it may have undesirable seed size or colour. These defects may be removed through the backcross method.

The nonrecurrent parent is selected for the character that is to be improved in the recurrent parent. The yielding ability and other characteristics of the nonrecurrent parent are not important. The only important factor in its choice is that it must have the character to be improved in an intense form. The intensity of the character should preferably be more than that desired in the recurrent parent

because some intensity of the character is generally lost during the transfer and in the new genetic background of the recurrent parent.

THE PROCEDURE OF BACKCROSS METHOD

The plan of the backcross method would depend upon whether the gene being transferred is recessive or dominant. The plan for transfer of a dominant gene is simpler than that for a recessive gene.

Transfer of a Dominant Gene

Let us suppose that a high yielding and widely adapted variety A is susceptible to stem rust. Another variety B is resistant to stem rust, and that resistance to stem rust is dominant to susceptibility. A generalised scheme of the backcross programme for the transfer of rust resistance from variety B to variety A is given below (Fig. 15.2).

Hybridization. Variety A is crossed to variety B. Generally, variety A should be used as the female parent. This would facilitate the identification of selfed plants, if any.

F_1 Generation. F_1 plants are backcrossed to variety A. Since all the F_1 plants will be heterozygous for rust resistance, selection for rust resistance is not necessary.

First Backcross Generation (BC_1). Half of the plants would be resistant and the remaining half would be susceptible to stem rust. Rust resistant plants are selected and backcrossed to variety A. BC_1 plants resistant to rust may be selected for their resemblance to variety A as well.

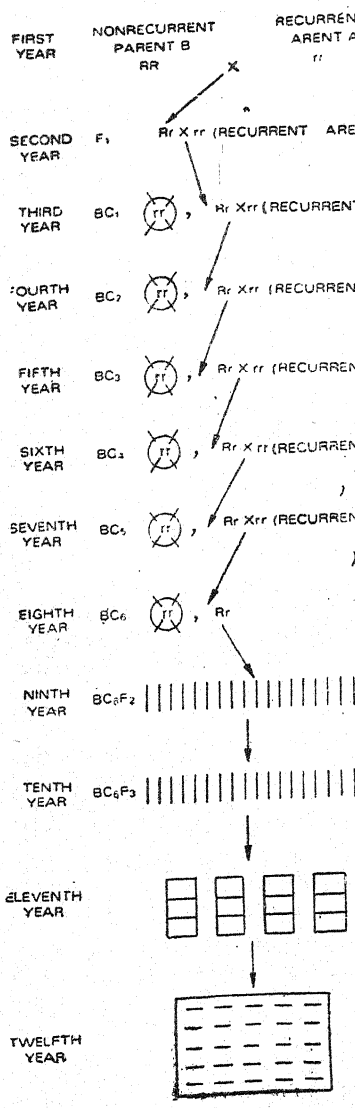
BC_2-BC_5 Generations. In each backcross generation, segregation would occur for rust resistance. Rust resistant plants are selected and backcrossed to the recurrent parent A. Selection for the plant type of variety A may be practised, particularly in BC_2 and BC_3.

BC_6 Generation. On an average, the plants will have 98.4 per cent genes from variety A. Rust resistant plants are selected and selfed; their seeds are harvested separately.

BC_6F_2 Generation. Individual plant progenies from the selfed seeds of the selected plants are grown. Rust resistant plants similar to the plant type of variety A are selected. The selected plants are harvested separately.

BC_6F_3 Generation. Individual plant progenies are grown. Progenies homozygous for rust resistance and similar to the plant type of variety A are harvested in bulk. Several similar progenies are mixed to constitute the new variety.

Yield Tests. The new variety is tested in a replicated yield trial along with the variety A as a check. Plant type, date of flowering, date of maturity, quality etc. are critically evaluated. Ordinarily, the new



B is the nonrecurrent parent, rust resistant but agronomically undesirable. A is the recurrent parent, rust susceptible, agronomically desirable.
F_1 backcrossed with the recurrent parent, variety A

(i) Rust resistant plants similar to variety A selected
(ii) Plants backcrossed with recurrent parent A

(i) As in BC_1

(i) As in BC_1

(i) As in BC_1

(i) As in BC_1

(i) Rust resistant plants self-pollinated
(ii) Seeds of selected plants harvested separately

(i) Individual plant progenies grown
(ii) Selection for rust resistance and plant type of A

(i) Individual plant progenies grown
(ii) Homozygous progenies similar to recurrent parent A harvested and bulked

Replicated yield trial with the recurrent parent as one of the checks

Seed multiplication for distribution

Fig. 15.2. A generalised scheme for the transfer of a dominant gene for disease resistance through the backcross method in a self-pollinated species.

variety would be identical to the variety A in performance. Detailed yield tests are, therefore, generally not required and the variety may directly be released for cultivation.

Transfer of a Recessive Gene

When rust resistance is due to a recessive gene, all the backcrosses cannot be made one after the other. After the first backcross, and after every two backcrosses, F_2 must be grown to identify rust resistant plants (Fig. 15.3). The F_1 and the backcross progenies are not inoculated with rust because they would be susceptible to rust. Only the F_2 is tested for rust resistance. A generalised scheme for the transfer of a recessive gene is given below (Fig. 15.3).

Hybridization. The recurrent parent is crossed with the rust resistant donor parent. The recurrent parent is generally used as the female parent.

F_1 Generation. F_1 plants are backcrossed to the recurrent parent.

BC_1 Generation. All the plants are self-pollinated. Since rust resistance is recessive, all the plants will be rust susceptible. Therefore, there is no test for rust resistance.

BC_1 F_2 Generation. Plants are inoculated with rust spores. Rust resistant plants are selected and backcrossed with the recurrent parent. Selection is done for the plant type and other characteristics of the variety A.

BC_2 Generation. There is no rust resistance test. Plants are selected for their resemblance to the recurrent parent A, and backcrossed with the recurrent parent.

BC_3 Generation. There is no disease test. The plants are self-pollinated to raise F_2. Selection is usually done for the plant type of variety A.

BC_3 F_2 Generation. Plants are inoculated with stem rust. Rust resistant plants resembling variety are selected and backcrossed to variety A. Selection for plant type of A is generally effective.

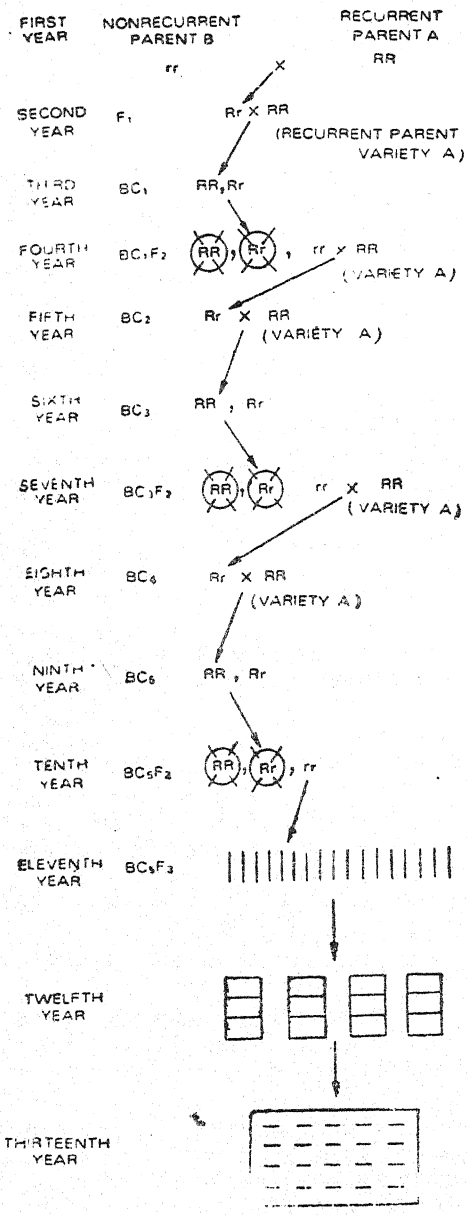
BC_4 Generation. There is no rust resistance test. Plants are backcrossed to variety A.

BC_5 Generation. There is no rust test. Plants are self-pollinated to raise F_2 generation.

BC_5 F_2 Generation. Plants are subjected to rust epidemic. A rigid selection is done for rust resistance and for the characteristics of variety A. Selfed seeds from the selected plants are harvested separately.

BC_5 F_3 Generation. Individual plant progenies are grown and subjected to rust epiphytotic. A rigid selection is done for resistance to stem rust and for the characteristics of variety A. Seeds from several similar rust resistant homogeneous progenies are mixed to constitute the new variety.

Yield Tests. It is the same as in the case of transfer of a dominant gene.



Variety B is rust resistant, variety A is susceptible but agronomically desirable
Varieties A and B crossed, B used as the female parent

(i) no rust resistance test
(ii) F1 backcrossed to the recurrent parent

(i) No rust resistance test
(ii) All plants are self-pollinated

(i) Rust resistance test
(ii) Resistant plants selected and (iii) Backcrossed to Variety A

(i) No rust resistance test
(ii) Plants backcrossed to Variety A

(i) No rust resistance test
(ii) All plants self-pollinated

(i) Rust resistance test
(ii) Resistant plants selected and (iii) Backcrossed with variety A

(i) No rust resistance test
(ii) Backcrossed to variety A

(i) No rust resistance test
(ii) All plants self-pollinated

(i) Rust resistance test
(ii) Resistant plants selected and harvested separately

(i) Individual plant progenies grown
(ii) Progenies similar to variety A selected and composited

Replicated yield test alogwith the variety A as check

Seed multiplication for distribution

Fig. 15.3. A generalised scheme for the transfer of a recessive gene for disesase resistance (in this case rust resistance) through the backcross method in a self-pollinated crop.

NUMBER OF PLANTS NECESSARY IN THE BACKCROSS GENERATIONS

According to the above schemes, only a few (about 10) seeds are necessary in each backcross generation for the transfer of a character governed by a single gene. This population size would almost certainly have at least one plant with the gene for rust resistance. However, if the character is governed by two or more genes, a larger number of backcross progenies would be required. A larger size of backcross population is also desirable to permit an effective selection for the plant type of the recurrent parent, and to increase the probability of recombination between the genes being transferred and the genes tightly linked with it. Therefore, more than 50, preferably 100 or more, plants should be grown in each backcross generation. In F_2 and F_3 generations, the population size should be as large as possible.

SELECTION FOR THE CHARACTER BEING TRANSFERRED

A rigid selection for the character being transferred must be practised during the backcross and the F_2 generations, otherwise the character is likely to be lost. It is, therefore, essential that the character being transferred must have a high heritability. Although monogenic characters are the easiest to transfer, the number of genes is not as important as the heritability of the character under transfer. It is desirable that the character should be easily identified either visually or through simple tests. The breeder should try to maintain the character in an intense form through selection. *Often some intensity would be lost due to modifying genes in the new genetic background. Therefore, the nonrecurrent parent should be chosen for a high intensity of the character to be transferred.*

NUMBER OF BACKCROSSES TO BE MADE

In the backcross method, it is essential that the genotype of the recurrent parent should be recovered except for the gene being transferred. The recurrent parent is likely to consist of several closely similar purelines. Therefore, a sufficient number of plants from the recurrent parent should be used for the backcrosses. This would make sure that the new variety will have the same genetic composition as the recurrent parent.

Generally, six backcrosses are sufficient to recover the essential features of the recurrent parent. Selection for the characteristics of the recurrent parent, particularly in the early backcross generations, may often have the effect of one or two additional backcrosses. *Thus six backcrosses alongwith selection for the recurrent parent plant type in the early backcross generations will be effective in recovering the genotype of the recurrent parent.*

TRANSFER OF LEAF RUST RESISTANCE TO MALVIYA 12 WHEAT

Malviya 12, a high yielding wheat variety, became highly susceptible to stem and leaf rusts within a couple of years of its release. Genes for resistance to these rusts were transferred from Sparrow through a backcross programme. Sparrow shows resistance to all the three rusts under artificial inoculation in the hot spots in India. In F_1 (Malviya 12 × Sparrow), 100 plants were grown ; all of them were backcrossed to Malviya 12. In BC_1, more than 500 plants were grown. Epidemic condition was created by planting infector rows of Agra Local, a wheat variety susceptible to all the known races of stem, leaf and stripe rusts. The inoculum consisted of the naturally occurring races of leaf and stem rusts ; no artificial inoculation was done. One hundred rust resistant plants were selected and backcrossed to Malviya 12. In each backcross generation, the plants were handled in the same way as in the BC_1. In BC_4, all the rust resistant plants (about 300) were selfed, and individual plant progenies were grown in BC_4F_2 ; 50 progenies homozygous for rust resistance were selected and their seeds were bulked to constitute the rust resistant Malviya 12. Yield trials revealed a slight (about 4%) improvement in the yielding ability of the new variety over the recurrent parent Malviya 12. During the backcross programme, the sole criterion of selection was rust resistance. One hundred rust resistant plants were selected in each generation to ensure that resistance to both the rusts (stem and leaf) was selected for, and that the resistant plants were not escapes since artificial inoculation was not used.

TRANSFER OF QUANTITATIVE CHARACTERS

Quantitative characters with moderate to high heritability, such as, grain size, plant height, flowering or maturity time etc., are suitable for transfer through the backcross method. *Invariably, some intensity of the character will be lost during the transfer. Therefore, the nonrecurrent parent must have the character in a more intense form than it is desired in the new variety.* After each backcross, the progeny are selfed and F_2 of 500-1000 plants is grown. A rigid selection for the character being transferred is practised in each F_2 generation. At the end of the backcross programme, the F_2 is handled according to the pedigree method. Several progenies homozygous for the character being transferred and similar to the recurrent parent in other characteristics are selected and mixed to make up the new variety. An example of such a transfer is provided by the medium grained rice variety Calady. Calady was developed through four backcrosses of the F_1 hybrid between Lady Wright (a long-grained variety) and Coloro (a short-grained variety) to Coloro. After each backcross, F_2 was grown and a rigid selection was practised for grain size.

TRANSFER OF TWO OR MORE CHARACTERS TO A SINGLE RECURRENT PARENT

When two or more characters are to be transferred to the same variety, one of the following three approaches may be used.

Simultaneous Transfer. The genes for the characteristics may be transferred simultaneously in the same backcross programme. The characters to be transferred are brought together in the hybrid by crossing each of the nonrecurrent parents to the recurrent parent or the hybrid thus produced. But in such a case, a larger backcross population would be needed than in the case of transfer of a single character. Further, the breeding programme may be delayed because the conditions necessary for the selection of all the characters may not occur every year. Sometimes, the two genes under transfer may be linked. In such a case, the transfer becomes very easy, and selection for only one gene may be necessary. Some examples of such a favourable linkage are between the genes *Lr 24* and *Sr 24*, *Lr 19* and *Sr 25*, and *Lr 26* and *Sr 31*.

Stepwise Transfer. The recurrent parent is first improved for one character. The improved recurrent parent is then used as the recurrent parent in a backcross programme for the transfer of the other character. If additional characters are to be transferred, they are transferred one at a time in a stepwise fashion. This approach takes much longer time for the transfer of two or more characters.

Simultaneous but Separate Transfers. Each character is tranferred to the same recurrent parent in simultaneous but separate backcross programmes. The resulting improved varieties from the different programmes are then crossed together. Homozygous lines for the characters being transferred are then selected from the segregating generations using the pedigree method. This approach appears to be the most suitable of the three methods.

MODIFICATIONS OF THE BACKCROSS METHOD

The backcross method may be modified in various ways to suit the needs of the breeder. Following are the three common modifications of the backcross method.

Production of F_2 and F_3. F_2 and F_3 generations are produced after the first and the third backcrosses. A rigid selection for the character being transferred and for the characteristics of the recurrect parent is done in the F_2 and F_3 generations. In the backcross progenies, selection need not be done either for the character being transferred or for the characteristics of the recurrent parent. The fourth, fifth and sixth backcrosses are made in succession. For the sixth backcross, a relatively larger number of plants from the backcross progeny are used. This method may be used for the transfer of both dominant and recessive genes. It is believed that an effective selection in F_2 and F_3 generations is equivalent to one or two additional backcrosses.

Use of Different Recurrent Parents. Often two or more good varieties have quantitative characteristics that are desirable in the new variety. These varieties may be used as recurrent parents in the same backcross programme. Each variety is used as a recurrent parent for one or two backcrosses. *The objective of this approach is to combine in the new variety some good genes from each of the recurrent parents with the genes from the nonrecurrent parent.* Noblisation of sugarcane is an outstanding example of this approach. Noble canes (*S. officinarum*) were first crossed with the Indian canes (*S. barberi*). The resulting hybrids were backcrossed to different varieties of noble canes to develop a large number of commercial sugarcane varieties. A similar approach was used for the transfer of scab resistance in apples and for the transfer of high vitamin C content from wild tomato (*Lycopersicon peruvianum*) to the cultivated tomato (*L. esculentum*).

Backcross-Pedigree Method. In this method, the hybrid is backcrossed 1-2 times to the recurrent parent. Subsequently, the backcross progeny are handled according to the pedigree method. This approach is useful when one of the parents is superior to the other in several characteristics but the noncurrent parent is not undesirable agronomically. The superior parent is used as the recurrent parent. The purpose of the one to two backcrosses is to make sure that the new variety would get a majority of the superior genes from the recurrent parent. It also leaves enough heterozygosity for transgressive segregants to appear. The varieties developed by this method must be put in yield trials as those developed by the pedigree method. The same holds true when two or more recurrent parents are used in the backcross programme.

APPLICATION OF THE BACKCROSS METHOD TO CROSS-POLLINATED CROPS

The backcross method is equally applicable to cross-pollinated crops. The method is essentially the same as in the case of self-pollinated crops. The only difference is that in cross-pollinated crops a large number of plants (100-300) from the recurrent parent must be used in each backcross. This is necessary so that the new variety has the same genetic constitution as the recurrent parent. For example, wilt resistance was transferred to alfalfa variety California Common from the variety Turkestan. Two hundred plants of California Common were used for each backcross. The new variety Caliverde is exactly like California Common except for its wilt resistance.

MERITS OF BACKCROSS METHOD

1. The genotype of the new variety is nearly identical with that of the recurrent parent, except for the genes transferred. Thus the outcome of a backcross programme is known beforehand, and it can be reproduced any time in the future.

2. It is not necessary to test the variety developed by the backcross method in extensive yield tests because the performance of the recurrent parent is already known. This may save upto 5 years time and a considerable expense.
3. The backcross programme is not dependent upon environment, except for that needed for the selection of the character under transfer. *Therefore, off-season murseries and green-houses can be used to grow 2-3 generations each year. This would drastically reduce the time required for developing the new variety.*
4. Much smaller populations are needed in the backcross method than in the case of pedigree method.
5. Defects, such as, susceptibility to disease, of a well-adapted variety can be removed without affecting its performance and adaptability. Such a variety is often preferred by the farmers and the industries to an entirely new variety because they know the recurrent variety well.
6. This is the only method for interspecific gene transfers.
7. It may be modified so that transgressive segregation may occur for quantitative characters.

DEMERITS OF BACKCROSS METHOD

1. The new variety generally cannot be superior to the recurrent parent, except for the character that is transferred.
2. Undesirable genes closely linked with the gene being transferred may also be transmitted to the new variety.
3. Hybridization has to be done for each backcross. This is often difficult, time taking and costly.
4. By the time the backcross programme improves it, the recurrent parent may have been replaced by other varieties superior in yelding ability and other characteristics.

ACHIEVEMENTS

The backcross method has been widely used in crop improvement. It has been used for transferring genes and chromosomes from one variety to another and from related species, and for the transfer of cytoplasm. Backcross method is the only breeding method suitable for the transfer of genes and chromosomes from one species to another. There are many examples of interspecific transfer of genes using the backcross method. 'Transfer' was the first commercial wheat variety in which rust resistance was transferred from a related species. For more examples of such a use of the backcross method, see Chapters 24 and 25. Two cotton varieties 170-Co 2 and 134-Co 2M, were developed using the backcross method. *Gossypium hirsutum* variety Dharwar-American 2-6-5 was crossed with *G. arboreum* variety Gaorani 6. The F_1 was highly sterile;

it set few seeds which gave rise to tetraploid plants (perhaps as a result of backcross to the *G. hirsutum* parent). Progeny from the F_1 were backcrossed to *G. hirsutum* varieties Dharwar-American (first backcross), Combodia Co 2 (second backcross) and Meade (third backcross). From the backcross progeny, 170-Co 2 and 134-Co 2M were selected These varieties had staple length of $1\frac{1}{4}$" (about 2.7 cm) and were widely cultivated in Gujarat both under irrigated and rainfed conditions.

The backcross method has been extensively used for the transfer of disease resistance to popular, widely adapted varieties. This is illustrated by the popular wheat (*T. aestivum*) variety Kalayan Sona, which became susceptible to leaf rust. Rust resistance has been transferred to Kalyan Sona from several diverse sources, *e.g*, Robin, K1, Bluebird, Tobari, Frecor, HS 19 etc., using the backcross method. Three multiline varieties, namely, KSML 3, MIKS 11 and KML 7406, have been released for cultivation as a result of the above. Another example is provided by the hybrid bajra varieties in India. The male sterile line Tift 23A (the female parent in the hybrids) was highly susceptible to downy mildew leading to a high susceptibility of the hybrids. Tift 23A was used in a backcross programme with resistant lines from India and Africa to develop downy mildew resistant male sterile lines, such as, MS 521, MS 541A, MS 570A etc., which are now being used for producing downy mildew resistant hybrids.

Characters other than disease resistance have been transferred by the backcross method. Good examples of this type are available in cotton. *G. herbaceum* varieties Vijapla, Vijay, Digvijay and Kalyan are some of the cotton varieties developed by the backcross method. From a variety Broach Local, a mixture of Goghari and Broach Desi varieties of *G. herbaceum*, two pureline varieties, Goghari A-26 and Broach Desi 8 (BD 8), were selected. Variety BD 8 was wilt resistant and had a high spinning value, but it had a low ginning outturn (34%). BD 8 was used as a recurrent parent in a backcross programme with Goghari A-26 which had a high ginning outturn (42-47%). High ginning outturn was transferred from Goghari A-26, the nonrecurrent parent, and a variety Vijay was developed. Vijay was further improved through the backcross method ; improved fibre length and early maturity were transferred from 1027A L-F (nonrecurrent parent). Variety Digvijay resulted from the above backcross programme.

COMPARISON BETWEEN BACKCROSS AND PEDIGREE METHODS

The two methods not only differ in their procedure and the system of mating for raising the generations after F_1, they differ in their end products as well. These differences are summarised in Table 15.2.

Table 15.2. Comparison between backcross and pedigree methods

	Pedigree method	*Backcross method*
1.	F_1 and the subsequent generations are allowed to self-pollinate.	F_1 and the subsequent generations are backcrossed to the recurrent parent.
2.	The new variety developed by this method is different from the parents in agronomic and other characteristics.	The new variety is identical with the recurrent parent, except for the character under transfer.
3.	The new variety has to be extensively tested before release.	Usually extensive testing is not necessary before release.
4.	The method aims at improving the yielding ability and other characteristics of the variety.	The method aims at improving specific defects of a well adapted, popular variety.
5.	It is useful in improving both qualitative and quantitative characters.	Useful for the transfer of both quantitative and qualitative characters provided they have high heritability.
6.	It is not suitable for gene transfer from related species and for producing substitution or addition lines.	It is the only useful method for gene transfers from related species and for producing addition and substitution lines.
7.	Hybridization is limited to the production of the F_1 generation.	Hybridization with the recurrent parent is necessary for producing every backcross generation.
8.	The F_2 and the subsequent generations are much larger than those in the backcross method.	The backcross generations are small and usually consist of 20-100 plants in each generation.
9.	The procedure is the same for both dominant and recessive genes.	The procedures for the transfer of dominant and recessive genes are different.

SUMMARY

In backcross method, the hybrid and the subsequent progeny are repeatedly backcrossed to one of the parents, the *recurrent parent*. The new variety is, therefore, the same as the recurrent parent, except for the character transferred from the other parent, the *nonrecurrent parent*. The recurrent parent is a well adapted variety, high in yielding ability and is popular among the cultivators. This variety is lacking in one or a few simply inherited characters. The nonrecurrent parent is selected for the intensity of the characters to be transferred. Backcross method is suitable for transfer of both qualitative and quantitative characters provided they have moderate to high heritability. Ordinarily, some intensity of the character would be lost during the transfer of the gene to the new genetic background of the recurrent parent. Backcross method is also applicable to cross-pollinated crops.

Generally, 6-7 backcrosses are necessary to recover the genotype of the recurrent parent. When two or more characters are to be transferred to a variety, each character should be transferred in separate backcross programmes. Finally, they are combined by crossing together the new varieties thus developed. Backcross method may be modified to allow transgressive segregation. The most common application of the backcross method has been in the transfer of disease resistance. It is also used for the transfer of qualitative

characters, qualitative characters, interspecific transfer of characters, transfer of cytoplasm, particularly for male sterility, and for the production of isogenic lines. The new varieties developed through complete backcrossing (6-8 backcrosses) generally do not require extensive yield trials for their release.

QUESTIONS

1. Define the following : recurrent parent, nonrecurrent parent, backcross, BC_1F_2, BC_2, B_1 and B_2.
2. List the requirements for a backcross programme. Describe the procedure of backcross method suitable for the transfer of a dominant gene for disease resistance.
3. Describe the procedure for backcross method for the transfer of a recessive gene. Discuss the merits and demerits of the backcross method of breeding.
4. Describe the applications of the backcross method and its achievements.
5. Write short notes on the following :

   (i) transfer of quantitative traits through backcross method, (ii) application of backcross method in cross-pollinated species, (iii) selection in backcross generations, (iv) modifications of backcross method, (v) number of plants necessary in a backcross generation, and (vi) number of backcrosses required in gene transfer programmes.

Suggested Further Reading

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CHAPTER 16

Other Approaches to Breeding of Self-Pollinated Crops

The methods of breeding described in chapters 11 to 15 are the common methods used for the improvement of self-pollinated crops. The objective of these methods, except mass selection, is to develop pureline varieties. These methods either use the variability already present in the population (mass selection and pureline selection) or the variability created through hybridization. The F_1 hybrid from a cross is either allowed to self-pollinate (pedigree and bulk methods) or is backcrossed to the desirable parent (backcross method). The effect of either of these approaches is a rapid increase in homozygosity. It is argued that the rapid increase in homozygosity reduces the chances of recovery of desired gene combinations, particularly when the genes are linked in repulsion phase.

A number of other breeding approaches have been suggested for the improvement of self-pollinated crops. Many of these approaches are modifications of the three basic schemes, *viz.*, pedigree, bulk and backcross methods. Usually two of these schemes have been combined to serve some specific purpose, *e.g.*, bulk-pedigree method, backcross-pedigree method etc. Clearly, these are not new approaches, but in certain situations they may serve some useful purpose. A discussion of these modifications is beyond the scope of this book. Three of the more important and new approaches are described below. These approaches are, (1) multiline varieties, (2) population approach and (3) hybrid varieties.

MULTILINE VARIETIES

Generally, pureline varieties are highly adapted to a limited area, but are generally poorly adapted to wider regions. Further, their performance is not stable from year to year because of changes

in weather and other environmental factors. Purelines often have only one or a few major genes for disease resistance, such as, rust resistance, which make them resistant to some races of the pathogen. New races are continuously produced in many pathogens which may overcome the resistance present in the pureline varieties. For example, Kalyan Sona wheat (*T. aestivum*) originally resistant to brown rust (leaf rust), soon became susceptible to the new races of the pathogen.

To overcome these limitations, particularly the breakdown of resistance to diseases, it was suggested to develop multiline varieties. *Multiline varieties are mixtures of several purelines of similar height, flowering and maturity dates, seed colour and agronomic characteristics, but having different genes for disease resistance.* The purelines constituting a multiline variety must be compatible, *i.e.*, they should not reduce the yielding ability of each other when grown in mixture. The idea of multiline varieties was put forward by Jenson in 1952 for use in cereals. In 1954, Borlaug suggested that several purelines with different resistance genes should be developed through backcross programmes using one recurrent parent. This is done by transferring disease resistance genes from several donor parents carrying different resistance genes to a single recurrent parent. Each donor parent is used in a separate backcross programme so that each line has a different resistance gene or genes. Five to ten of these lines may be mixed to produce a multiline variety. Which lines are to be mixed would be determined by the races of the pathogen prevalent in the area. If a line or lines become susceptible, they would be replaced by resistant lines. New lines would be developed when new sources of resistance become available. The breeder should keep several resistant lines in store for future use in the replacement of susceptible lines of multiline varieties.

Merits of Multiline Varieties

1. All the lines are almost identical to the recurrent parent in agronomic characteristics, quality etc. Therefore, the disadvantages of the pureline mixtures (Chapter 11) are not present in the multiline varieties.

2. Only one or a few lines of the mixture would become susceptible of the pathogen in any one season. Therefore, the loss to the cultivator would be relatively low.

3. The susceptible line would constitute only a small proportion of the plants in the field. Therefore, only a small proportion of the plants would be infected by the pathogen. Consequently, the disease would spread more slowly than when the entire population was susceptible. This would reduce the damage to the susceptible line as well.

Demerits of Multiline Varieties

1. The farmer has to change the seed of multiline varieties every few years depending upon the change in the races of the pathogen.
2. There is a possibility that a new race may attack all the lines of a multiline variety.

Achievements

Multiline variety appears to be a useful approach to control diseases like rusts where new races are continuously produced. In India, three multiline varieties have been released in wheat (*T. aestivum*). Kalyan Sona, one of the most popular varieties in the late sixties, was used as the recurrent parent to produce these varieties. Variety 'KSML 3' consists of 8 lines having rust resistance genes from Robin, Ghanate, K 1, Rend, Gabato, Blue Bird, Tobari etc. Multiline 'MIKS 11' is also a mixture of 8 lines ; the resistance is derived from E 6254, E 6056, E 5868, Frecor, HS 19, E 4894 etc. The third variety, KML 7406 has 9 lines deriving rust resistance from different sources.

POPULATION APPROACH TO BREEDING OF SELF-POLLINATED CROPS

Self-fertilization of F_1 hybrids leads to a very rapid increase in homozygosity. After only 4 generations of self-pollination, about 94 per cent of the genes would become homozygous. Even in F_2, half of the genes are in homozygous state. Thus self-fertilization quickly separates the progeny from a hybrid into a large number of purelines. As a consequence, selection in such a segregating population only picks out the gene combinations present in the population primarily as a result of recombination in F_2. This reduces the chances of recombination between linked, especially tighty linked, genes and of recovery of rare transgressive segregants. There is no opportunity for changing the genotype of the plant produced by recombination in F_1, F_2 and, to some extent, in F_3. Thus the two obvious limitations of breeding methods based on self-pollination of the hybrid (*e.g.*, pedigree and bulk methods) are: *first*, the recombination is limited to two or, at best, three generations, and *second*, there is no possibility for further changing the genotype of the segregants.

A population breeding approach has been suggested to overcome these problems. In population breeding, outstanding F_2 plants are mated among themselves in pairs or in some other fashion. The intermating of selected F_2 plants restores heterozygosity in the progeny, which provides for a greater opportunity for recombination. This also brings together the desirable genes from different F_2 plants and would help in the accumulation of favourable genes in the intermated population. Thus the chances of the recovery of transgressive

segregants would increase considerably. This process may be repeated one or more time (Fig. 16.1). This procedure is similar to recurrent selection in cross-pollinated crops (Chapter 18). A variation of this approach would be to intermate F_3 or later generation progenies. This would allow a more effective selection of desirable progenies than in the case of F_2 where individual plants have to be selected. As noted previously, selection in F_2 based on individual plants is of little value, particularly for characters like yield. Selection based on

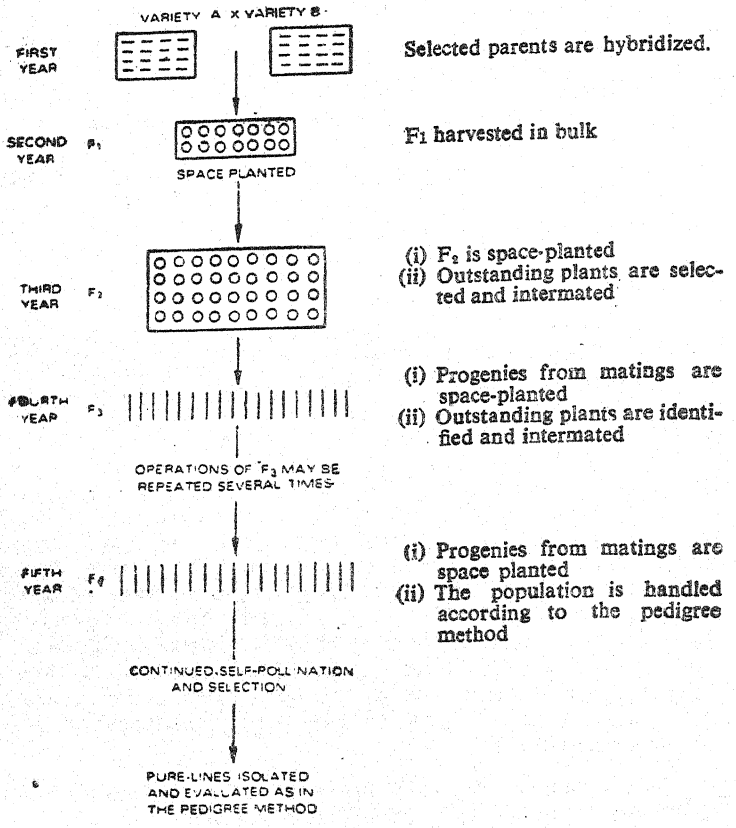


Fig. 16.1. Schematic representation of the population approach to breeding of self-pollinated crops.

F_3 or F_4 progenies would be more desirable. Intermating of selected plants may be continuted for two or more generations.

The idea of population approach was first suggested by Palmer in 1953. It is not commonly used at present, but may find a greater application in the future as improvements due to the pedigree method would become less and less marked. Evidently, the population approach is akin to *recurrent selection* commonly used in cross-pollinated crops (Chapter 18), and often it is referred to as such. The chief limitation of recurrent selection in self-pollinated crops is the difficulty in making the large number of required crosses by hand (emasculation and pollination). This difficulty may be overcome by using genetic or cytoplasmic male sterility. When genetic male sterility is used, selection is confined to the male sterile (*ms ms*) plants in each generation. Seeds from the selected male sterile plants are generally harvested in bulk. The progeny from such plants may be expected to have both male sterile (*ms ms*) and male fertile (*Ms ms*) plants in almost equal proportion. Further, the seeds produced on the male sterile plants would be produced by pollination by the male fertile plants in the population. Thus the use of male sterility effectively ensures intermating among the plants of a population and eliminates the need for tedius and time consuming hand emasculation and pollination.

Results from recurrent selection are available in tobacco and soybean. In tobacco, Matzinger and coworkers selected the plants before flowering and intermated them. A linear response of 4.9 and 7 per cent per cycle to selection for decreased plant height and for increased leaf number, respectively, was obtained for five cycles of selection. Further, there was no evidence for a reduction in variability as a result of the selection. Brim and coworkers carried out six cycles of recurrent selection for increased protein content in two segregating populations of soybean, and three cycles of selection for yield and three cycles of selection for high oil content in another segregating population. There was an increase of 0.33 and 0.67 per cent/cycle in protein content of the two populations, of 5.3% per cycle in yield and of 0.3% per cycle in oil content. These findings amply demonstrate the effectiveness of recurrent selection in improving yield and yield traits in self-pollinated crops.

In 1970, Jensen proposed a comprehensive breeding scheme which provides for the three basic functions of a versatile breeding programme. *Firstly*, it allows the development of F_2, F_3 etc. (*selfing series*) at every stage of the breeding programme, which permits the isolation of purelines for use as commercial varieties. *Secondly*, it requires intermating among the selected plants/lines in each stage; the progenies from these intermatings form the basis for the next stage of the selfing series in the breeding programme. Thus the breeding programme progresses in two different directions : (1) *Vertically*, through the selfing series leading to the isolation of commercial varieties, and (2) *horizontally*, through intermating

among the selected plants/lines ; this generates the *recurrent selection series*. *Thirdly*, new germplasm may be introduced at any stage of the programme by intermating it with some of the selected plants of that stage. This permits the retention and/or the creation of large amounts of variability for effective selection through several cycles, and the introduction of new genes in the breeding material, if so desired. This breeding scheme is known as *Diallel Selective Mating Scheme* (DSM) and is designed to serve both short-term and long-term breeding objectives (Fig. 16.2). A breeder may create more than one such population for a crop, each population being developed to fulfil a specific objective. This scheme has not been widely used primarily due to the difficulties in making the large number of crosses required in this scheme. Jensen has suggested the use of male sterility to overcome this difficulty in the same way as in the recurrent selection scheme discussed earlier. Further, DSM is much more complicated than the simple pedigree method which still is the favourite breeding method for self-pollinated crops.

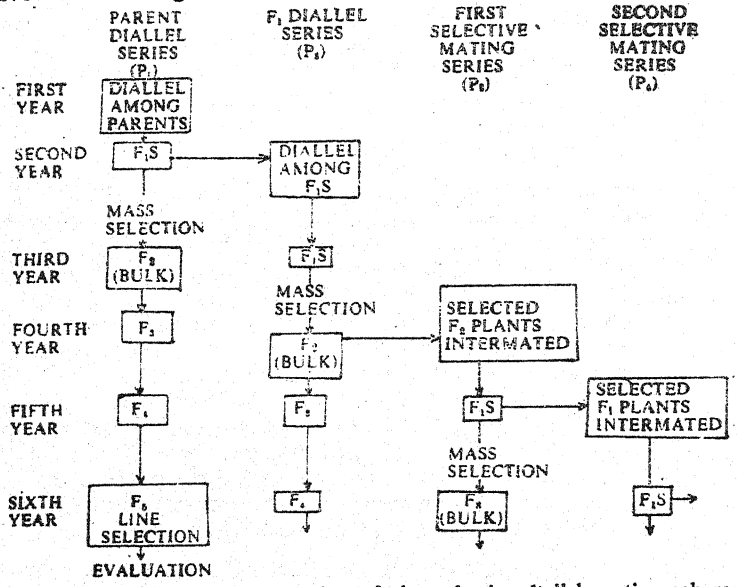


Fig. 16.2. A schematic representation of the selective diallel mating scheme. The seed produced by F_1 plants in each (P_1, P_2, P_3, P_4 etc.) series are subjected to mass selection for seed characteristics, and the selected seeds from all the crosses in a series are composited to establish a bulk F_2 population. The F_2 and subsequent generations may be handled according to bulk, pedigree or single seed descent schemes. New germplasm may be introduced in any of the P_2, P_3, P_4 or subsequent series by mating the new lines with some of the selected plants from the previous series, that normally serve as parents for intermatings to generate the concerned series.

Merits of Population Approach

1. The population approach provides for geater opportunities for recombination. This is made possible by restoring heterozygosity through intermating of selected plants.
2. This approach helps in the accumulation of desirable genes in the population. This also is brought about by the intermating of selected plants from segregating generations.

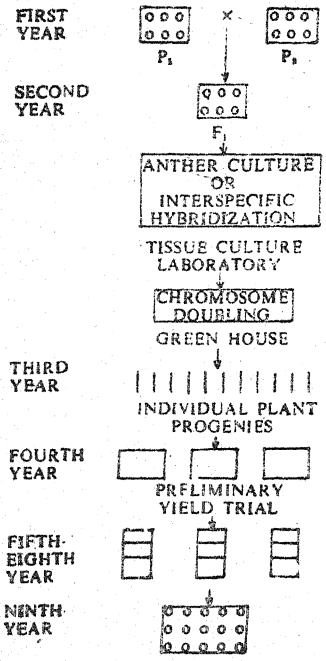
Demerits of Population Approach

1. The success of this approach depends upon the identification of desirable plants in F_2 and the subsequent segregating generations. This is very difficult, if not impossible, for complex characters like yield which show low heritability. This may be avoided to some extent by using later generation (F_3 or F_4) progenies ; replicated yield data may also be used.
2. Another drawback of this approach is the intermating of selected plants. This may become a major limitation in some crops because crossing in many self-pollinated species is difficult and time consuming.
3. The time taken to develop a new variety through population approach would be always greater than that by the pedigree method.
4. There is no convincing evidence for the benefits from the population approach. It has been argued that increased recombination may be detrimental as it would break the desirable linkages. But such a criticism assumes that all or most of the new gene combinations (recombinations) will be inferior to the existing ones. Such an assumption is not entirely valid since crop improvement is based on the creation of new and desirable gene combinations.

RAPID ISOLATION OF HOMOZYGOUS LINES

In self-pollinated crops, the breeders aim at developing superior homozygous lines. In crossing programmes, the segregating materials have to be carried to at least F_5 or, often, F_6 before the lines become reasonably homozygous to permit preliminary evaluation. The progress under selection upto this stage is hampered by heterozygosity and it takes 4-5 years to obtain partially homozygous lines. Techniques are now available, at least in some crop species, for the isolation of completely homozygous lines from the F_1 generation itself. This technique consists of (1) extraction of haploid plants from F_1 plants using anther culture or distant hybridization, and (2) chromosome doubling of such haploid plants to obtain completely homozygous diploid plants and, subsequently, progenies.

A generalized scheme for rapid isolation of homozygous lines is outlined in Fig. 16.3. Selected parents are hybridized (*First year*).



Selected lines are mated

F_1 seeds space-planted, preferably under controlled environment

(i) Anthers from F_1 plants cultured on an appropriate culture medium
(ii) Haploid plants regenerated.

(i) Regenerated plants planted in a green houses
(ii) Chromosome doubling through colchicine or some other agent
(iii) Seeds from individuals plants harvested separately

(i) Individual plant progenies grown in field
(ii) Superior progenies harvested seperately

(i) Preliminary yield trial with suitable checks.
(ii) Outstanding lines included in co-ordinated yield trials

(i) Multilocation yields trials with suitable checks.
(ii) Outstanding progeny released as a new variety

Seed increase for distribution to farmers

Fig. 16.3. Production of homozygous lines through anther culture or interspecific hybridization in breeding of self-pollinated crops.

and the F_1 generation is space-planted (*second year*). It may be desirable to grow the F_1 under controlled conditions since the environment is likely to influence the success of anther culture from these plants. Anthers of an appropriate developmental stage from the F_1 plants are inoculated on a suitable culture medium (see Chapter 26) in order to ultimately obtain haploid plants either through direct embryogenesis from pollen grains or through callus and, later, plantlet development from pollen grains. The haploid plants are finally transferred to soil and are subjected to, ordinarily, colchicine treatment in order to double their chromosome number. It is essential to standardise both anther culture and chromosome doubling techniques in order to maximise the recovery of haploid and doubled haploid (homozygous diploid) plants, respectively. Haploids may be extracted from F_1 plants through a suitable distant hybridization. In barley, this is achieved by crossing the F_1 plants to *Hordeum bulbosum* ; the young embryos from this cross are generally raised through embryo culture.

Seeds from individual doubled haploid plants are harvested separately and are used to plant individual plant progeny rows in the *third year*. The individual plant progenies may be planted in an *augmented design* (single rows of progenies in an unreplicated trial with a suitable check variety recurring every third, fifth, seventh or eleventh row) to facilitate the elimination of weaker progenies by comparing them with the contiguous control or the moving average. In the *fourth year*, superior progenies are planted in a preliminary yield trial having suitable checks. Outstanding progenies are identified and evaluated in multilocation yield trials under the concerned all India coordinated crop improvement project from *fifth* to *eight years*. The outstanding progeny, if any, may be released as a new variety and its seeds multiplied and distributed to farmers in the *ninth year*.

This represents a saving of at least 3-4 years in the development of a new variety. But the availability of a well equipped tissue culture laboratory and dependable green house facilities are a prerequisite for this technique to be of practical importance. Off-season nurseries may be used as a substitute for green-houses, but this would involve considerable transport and travel efforts, which in most cases may be prohibitive in view of the expected gains. For a few crop species, areas may exist where two or more crops are taken one after the other, *e.g.*, rice in parts of Orissa, Tamil Nadu, Kerala etc. Such special situations may be exploited where they exist, but for most crops the necessity of green house facilities is a prerequisite. Another limitation of the technique is the limited number of crops for which reliable techniques for haploid production are available ; further research efforts are likely to enlarge the list of such crops in the future.

In this method, homozygosity is achieved in a single generation, while it may often be desirable to maintain heterozygosity for several generations to permit rare recombinations to occur. The breeder may combine the technique for rapid isolation of homozygous lines with a suitable scheme for population improvement or recurrent selection to permit a rapid recovery of homozygous lines at the end of selection period (Fig. 16.4).

HYBRID VARIETIES

Self- and often cross-pollinated crops show little or no loss in vigour or yield due to inbreeding. But F_1 hybrids in such crops are generally more vigorous and higher yielding than either of their parents. They are also more stable phenotypically than the parental purelines. The superiority of an F_1 over its parents is known as *heterosis* or *hybrid vigour*. Heterosis is commercially utilised by using F_1 hybrids as commercial varieties, *i.e.*, hybrid varieties. Hybrid varieties are much more common in cross-pollinated species than in self-pollinated species for two obvious reasons. *Firstly*, cross-pollinated crops are well suited for the production of large quantities of hybrid seed since they are naturally cross-pollinated.

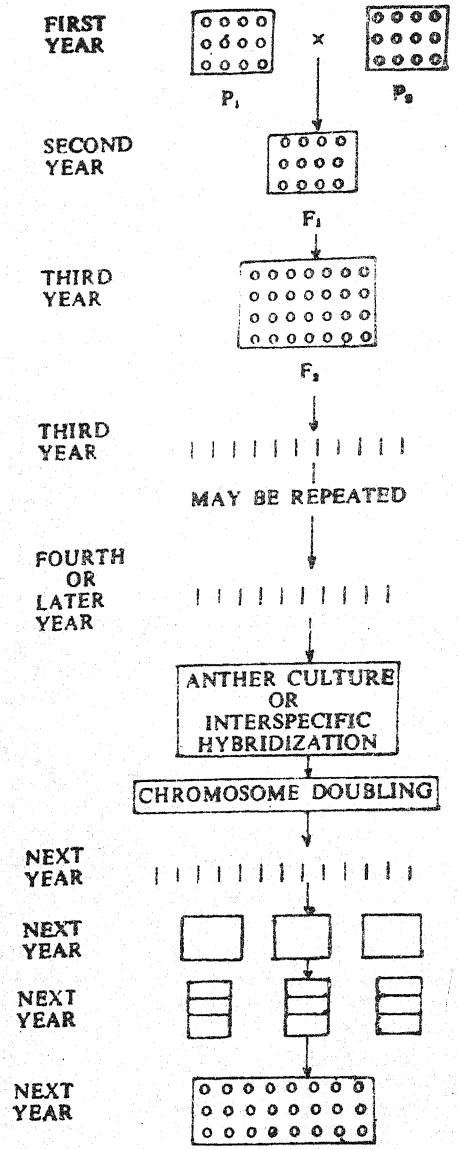


Fig. 16.4 Production of homozygous lines through anther culture or interspecific hybridization after one or more generations of intermating of the selected plants in self-pollinated crops.

As a result, the cost of hybrid seed production is comparatively very low in cross-pollinated than in self-pollinated species. *Secondly*, in self-pollinated crops superior pureline varieties give considerably high yield and they are easily multiplied and maintained. But in cross-pollinated crops heterozygosity must be maintained to prevent inbreeding depression. In spite of these, hybrid varieties have been used commercially in some self-pollinated crops like tomato, brinjal, tobacco, cotton and jowar.

Many F_1 hybrids in wheat (*T. aestivum*) give 25-30 per cent higher yields than the average of the two parents, while in jowar (*S. bicolor*) the increase in yield may be as high as 25-40 per cent. Outstanding hybrids in jowar may outyield the standard varieties by a margin of 50-80 per cent. Hybrid vigour is known in almost all the self-pollinated crops. But in many crops it has not been possible to utilize heterosis commercially because the production of large quantities of hybrid seed in these crops is very costly and often extremely difficult. The hybrid seed is produced by using cytoplasmic-genetic male sterility or by hand pollination.

Use of Cytoplasmic-Genetic Male Sterility. Cytoplasmic male sterility is known in many self-pollinated crops, *e.g.*, wheat, cotton (*Gossypium* sp.), tobacco (*Nicotiana* sp.), sorghum (*S. bicolor*), chilles (*Capsicum annuum*) etc. But in most of the cases, it is not suitable for hybrid seed production due to one or more of the following reasons.

1. The male sterile cytoplasm has undesirable side effects, *e.g.*, tobacco (*Nicotiana* sp.).
2. Effective restorer genes are not available, *e.g.*, cotton (*Gossypium* sp.).
3. Natural cross-pollination and seed set in the male sterile line is not satisfactory, *e.g.*, chillies (*Capsicum annuum*).
4. The male sterile cytoplasm may produce incomplete male sterility.

Cytoplasmic-genetic male sterility is commercially used in jowar to produce hybrid seed. A similar system is available in wheat, but it is not yet commercially exploited. The cytoplasmic male sterility in jowar was discovered in 1950 when the chromosomes of a *kafir* variety were transferred in the cytoplasm of a *milo* variety. A male sterile line thus produced, *viz.*, Combine Kafir 60 MS, has been commonly used for hybrid seed production in jowar. The fertility is restored by a dominant gene *Msc*, which occurs mostly in *milo* varieties or in varieties of *milo* origin. The production of hybrid seed involves the following steps (Fig. 16.5).

(*a*) Development of Male Sterile Lines. Hybrid seed production may utilise the already existing male sterile lines, known as A lines, *e.g.*, Combine Kafir 60 MS in jowar. New male sterile lines may be developed by backcrossing the male sterile hybrid from a cross between a male sterile line (used as female) and a male fertile line (used

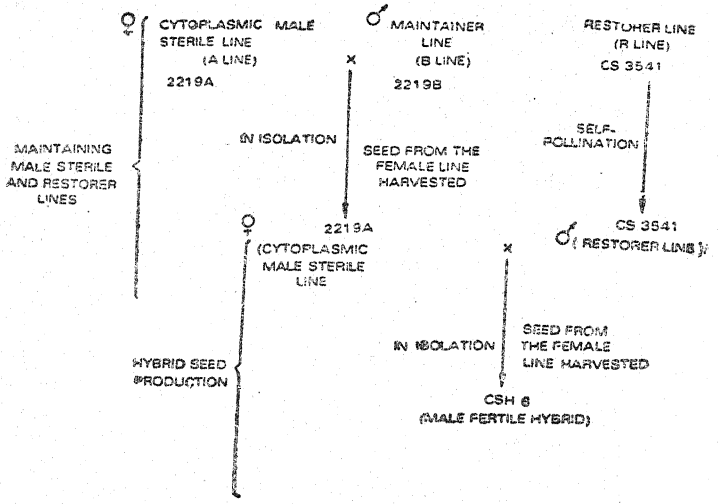


Fig. 16.5. Scheme for producing hybrid jowar using cytoplasmic-genetic male sterility.

as male), to the male parent. About 6-8 backcrosses would be sufficient to obtain all the genes of the male fertile line in the male sterile cytoplasm. The new male sterile line, the *A line*, will be identical with the male fertile line, known as *B line* or maintainer line, used in the backcross programme.

(*b*) **Maintenance of Male Sterile Lines.** The male sterile lines (*A lines*) are maintained by crossing them with the male fertile lines (*B lines*) from which they were developed by backcrossing. The B lines are known as maintainer lines because they are used for maintaining the male sterile lines. Thus the male sterile lines are maintained by continuously backcrossing them with their male fertile parent.

(*c*) **Production of Hybrid Seed.** For production of hybrid seed, the male sterile line is pollinated with the pollen from a restorer line. The restorer line carries the gene or genes for restoring male fertility in the presence of male sterile cytoplasm. It must combine well with the male sterile line to produce a high yielding and desirable hybrid. In jowar, generally six rows of the male sterile line are planted with two lines of the restorer line. Seeds from the male sterile line are harvested since they are hybrid in origin.

Several outstanding hybrids have been developed in jowar using cytoplasmic-genetic male sterility. Some of the hybrids released for cultivation are : CSH 1, CSH 2, CSH 3, CSH 4, and CSH 5 and CSH 6 etc. CSH stands for *coordinated sorghum hybrid.*

Hand Pollination. Production of hybrid seed by hand pollination is commercially feasible in crops like tomato (*Lycopersicon esculentum*), brinjal (*Solanum melongena*) and tobacco because each fruit in these crops produces a large number of seeds. For example, one fruit in brinjal and tobacco produces about 2,500 seeds. Hybrid tomatoes produced by hand pollination are commercially grown in Europe. In India, hybrid cotton is produced by hand pollination and is commercially grown at a large scale. The area under hybrid cotton is currently more than one million hectares. Hybrid cotton is produced by emasculating the flowers of one variety and pollinating the emasculated flowers with pollen grains from the other parent variety. Emasculation is done one day before the flower would normally open and the flowers are pollinated immediately after emasculation. For emasculation, the corolla is cut away with a pair of scissors and the anthers are removed with a pair of wide-tip forceps. Pollen is collected in a small piece of soda straw. The soda straw is then slipped over the stigma of the emasculated flower. The bracts are pulled up and tied together to hold the soda straw in place. This technique generally gives about 75 per cent seed set.

Several hybrid cotton varieties have been released for commercial cultivation ; hybrid varieties obtained by crossing two different strains of *G. hirsutum* are H 4 (H=hybrid), JKHy 1, Godavari (NHH 1), Suguna, H 6 and AHH 468 (PKVHy 2), while those derived by crossing one strain of *G. hirsutum* with a strain of *G. barbadense* are Varalaxmi, CBS 156, Savitri (RHR 253), Jaylaxmi (DCH 32) and K 2HC. H 4 was the first hybrid variety developed by the Gujarat Agriculture University (Surat station) and released in 1970. Varalaxmi was the first interspecific hybrid variety developed by the University of Agricultural Sciences (Dharwad station) and released in 1972. India is the first country to commercially exploit hybrid vigour in cotton. All the hybrid varieties have been developed in the tertaploid cottons and no hybrid variety has been released in the diploid cottons. These hybrids yield 30-40 q/ha seed cotton with 32-36.5% ginning outturn ; the fiber length ranges from 24.5 to 33 mm, *i.e.*, they have extra long fiber. In addition, a male sterile line Gregg is being used for hybrid seed production. All India Coordinated Cotton Improvement Project is extensively using this male sterile line to reduce the cost of hybrid seed. Several varieties developed in this manner are in the various stages of testing prior to their release for commercial cultivation.

SUMMARY

In self-pllinated crops, multiline varieties may be used to reduce losses due to diseases like rusts in wheat. Intermating of selected F_2 or later generation plants from a cross would help in obtaining rare recombinations and in accumulating favourable genes. Such a population approach to breeding of self-pollinated crops may become increasingly important in future. In many self-pollinated crops, *e.g.*, cotton, jowar, tomato, rice etc., F_1 hybrids are used as varieties. The hybrid varieties may be produced by hand pollination, *e.g.*, in cotton, tomato, tobacco, etc., or by cytoplasmic-genetic male sterility, *e.g.*,

in jowar, and rice. Heterosis is common in almost all the self-pollinated crops. But hybrid varieties are not used in most of them due to a very high cost of hybrid seed, and due to a great difficulty in obtaing large quantities of the hybrid seed.

QUESTIONS

1. Differentiate between the following :
   (i) multilines and mixed populations, (ii) multiline varieties and varieties developed through mass selection, (iii) population approach and hybrid varieties, and (iv) restorer and maintainer lines.
2. Define multilines. Discuss their production, uses, merits and demerits. Describe briefly the achievements through multilines in India.
3. Briefly describe the contribution of the following scientists : Jenson, Borlaug and Palmer.
4. What breeding methods would you suggest in the following situations ?
   (i) tight linkage between desirable and undesirable traits, (ii) a crop species is attacked by a disease that has many physiological races, (iii) the character under improvement has low heritability, (iv) the character is mainly governed by additive gene action, and (v) the character is chiefly governed by nonadditive gene action.
5. Discuss the relative importance of the following breeding methods :
   (i) multiline and the population approach, (ii) multiline and hybrid varieties.
6. Discuss the scope of hybrid varieties in self-pollinated crops. Briefly describe the method of production of hybrid varieties in cotton and jowar.
7. What is the population approach of breeding self-pollinated crops ? Describe a generalised procedure for population approach to breeding and discuss its merits and demerits.

Suggested Further Reading

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CHAPTER 17

Effectiveness of the Different Breeding Methods for Self-Pollinated Crops

The mass and purelines selection methods are applied to genetically variable but homozygous populations of self-pollinated crops ; they are not designed for the handling of segregating generations obtained from crosses. The backcross method has well-defined objectives, definite requirements and a predictable outcome. Therefore, it is not suited for a general application to the segregating generations from crosses, although it can be combined with other breeding methods, *e.g.*, pedigree, to suit specific needs. Of the remaining two main breeding methods for self-pollinated crops, the pedigree method (PS) is by far the most widely used, while the bulk procedure (BP) has remained something of a curiosity for most breeders. The main objective of both these breeding methods is the isolation of superior purelines from segregating generations of crosses, the plants being allowed to reproduce by selfing. Two alternative techniques for obtaining purelines from crosses, *viz.*, doubled haploid (DH) and single seed descent (SSD), are now available ; these techniques are much more rapid and require relatively less effort and expenditure. It is, therefore, pertinent to examine the relative effectiveness of these techniques in order to afford a logical and emperical choice for an efficient and less demanding breeding procedure for self-pollinated crops.

PEDIGREE METHOD

At present, the pedigree method is the most extensively used. In India, this method appears to be the method of choice for all the breeders of self-pollinated crops. The success of this method is evident from the number of improved varieties it has yielded. However, this fact does not necessarily reflect the relative efficiency of the procedure. The large number of varieties due to PS may simply be due to its extensive use almost to the exclusion of other methods. Further, the overwhelming popularity enjoyed by PS may be due to reasons other than its effectiveness. For example, (i) it is the earliest devised method for the handling of segregating generations from crosses. (2) It provides an opportunity to the breeders for the selection of 'superior' and 'desirable' types beginning from the earliest segregating generation, that is, F_2. (3) Breeders get the

satisfaction of applying their 'skill' from F_2 onward, *i.e.*, they are active participants in the breeding programme. In addition, (4) the method is no doubt highly effective in changing qualitative characters and highly heritable quantitative characters in the direction of selection. However, the crucial question is, 'is selection during the segregating generations for quantitative characters with moderate to low heritability effective ?'

Conventionally, visual selection is the basis of PS, but doubts have been expressed about its efficacy (Shebeski, 1967). Some authors concluded that a visual selection for superior yielding lines was ineffective as there was a poor agreement between visual ratings and actual yields of lines (see Boerma and Cooper, 1975). Shebeski (1967) advocated the use of contiguous control for early generation yield testing (early testing) of individual plant progenies. It has been reported that an analysis of covariance is likely to increase the effectiveness of contiguous control technique in the identification of superior yielding progenies. But the reports on the usefulness of early testing are conflicting. Some workers have reported that early testing is able to identify higher yielding lines, while others found it to be ineffective (see, Salmon *et al.*, 1978 ; Boerma and Cooper, 1975 ; Knott and Kumar, 1975).

Estimates of correlation between yields of different generations have been used as a measure of the effectiveness of visual and early testing selections. There is a considerable variation among the correlation estimates reported from different studies. In general, significant correlations between yields of F_3 lines and those of later generation lines derived from them have been observed but in some studies the correlation estimates were nonsignificant. Further, it is doubtful that the significant correlations (even if $r = 0.60$) justify the effort and expenditure involved in early testing (see, Knott and Kumar, 1975).

In case of wheat, McGinnis and Shebeski (1968) reported that the identification of high yielding F_2 plants by these selectors was successful, but the correlations between F_2 plant yields and F_3 plot yields in the following season were not significant. In other similar studies, visual selection among F_3 lines resulted in an yield increase, but this increase was relatively smaller as compared to that from selection based on plot yields (see, Salmon *et al.* 1978). Salmon *et al.* (1978) developed low yielding, high yielding, random and visual F_4 bulks in four crosses of triticale. These bulks were made up of F_3 lines selected for low yield, for high yield, randomly and visually, respectively, and were evaluated at two locations. The yields of visual and high yielding bulks were comparable in the four crosses at both the locations. In most of the cases, the random bulk, although numerically inferior, was also comparable to the visual and high yielding bulks (Table 17.1). Clearly, early testing in F_3 was not superior to visual selection (pedigree) in the identification

Table 17.1. Mean yields of F_4 bulks derived from four crosses of triticale at two locations (Glenlea-Carman ; combined) in Manitoba, Canada and one location (Ciano) in Mexico (based on Salmon *et al.*, 1978)

Selection group bulks	*Mean yield (g/plot)*	
	Glenlea-Carman	*Ciano*
83 × Koala-3		
Low yield bulk**	135.4a*	426.7a
Random selection bulk	144.9a	445.7a
Visual selection bulk	187.7ab	573.2b
High yield bulk**	211.6b	563.5b
(Maya II × Armadillo²) × 6 TA 204		
Low yield bulk	118.0a	473.2a
Random selection bulk	187.6ab	512.0a
Visual bulk	237.2b	631.5b
High yield bulk	233.1b	568.7b
(6 TA 204 × Bronco 90) × (Aa...ver × Brmadillo)		
Low yield bulk	129.9a	338.5a
Random selection bulk	160.9ab	437.0b
Visual bulk	188.9b	583.0c
High yield bulk	186.0b	497.2b
Koala-3 × 6¦TA 518		
Low yield bulk	270.0a	494.0a
Random selection bulk	258.7a	577.5ab
Visual bulk	271.2a	651.3b
High yield bulk	279.2a	640.3b

* Values followed by the same letter are not significantly different, comparisons within crosses within locations.

** Low yield and high yield bulks developed on the basis of yield data on F_3 lines.

of superior yielding lines of triticale. However, early testing effectively identified poor yielding F_3 lines.

It emerges from the foregoing that visual selection in early segregating generations of crosses appears to be as effective in the identification of high yielding lines/plots as actual yield tests (early testing). This raises an important question of cost and benefit on the usefulness of early testing. Another major disadvantage of early testing is that it restricts the number of progenies that can be evaluated. Further, there is a risk in early testing of rejecting low yielding lines which may generate desirable recombinants in the subsequent generations ; in fact, this has been suggested as one of the serious weakness for PS as well (see later).

SINGLE SEED DESCENT

The idea of single seed descent (SSD) was originally suggested by Goulden in 1939 and subsequently modified by Brim in 1966. Brim referred to this procedure as modified pedigree method, and this name has often been used in the literature (see, Snape and Riggs, 1975). However, the procedure lacks both the essential features of PS, *viz.*, maintenance of pedigree records, and individual plant selection during the segregating generations. In all respects, it is essentially a variation of the bulk procedure, and should be regarded as such (Tee and Qualset, 1975). The method has aroused considerable interest among plant breeders and offers an unique opportunity for rapidly advancing segregating generations. The merit of the method is evident from both theoretical consideration and experimental findings, as well as from its ever increasing popularity among the breeders.

Theoretical Considerations. Theoretically, the mean expression of a character in F_1 and its distribution in F_2 will depend on genetic architecture of the character, but the distribution of F_6 lines obtained through SSD will be more or less the same irrespective of the genetic situation (Snape and Riggs, 1975). In the absence of dominance, the F_2 and F_6 genotypic distributions will be similar, but more of the F_6 lines will be as good as the extreme homozygote, that is, the homozygote containing all the positive alleles for the trait. But when a large amount of directional dominance is exhibited, the F_6 distribution will fall markedly on the lower side of F_2 distribution, and only about 2% of the F_6 lines will exceed the F_2 mean. Similar results are expected in the case of complementary epistasis. In the presence of duplicate epistasis, the F_2 distribution will be markedly skewed to the right (higher expression of the trait) and about 40% of the individuals will have the phenotype of the extreme homozygote. The distribution of F_6 lines will be much less skewed and will be similar to that in the absence of dominance, but only about 6% of the F_6 lines will be comparable to the extreme homozygote. Further, the genetic advance expected from PS and SSD will be comparable if there is no dominance or in the presence of duplicate epistasis, but in the presence of marked directional dominance or complementary epistasis the former will be superior to the latter.

Cockerham and Matzinger (1985) evaluated the effects of the number of generations of selfing on selection response in SSD populations. They used a quantitative genetic model for an arbitrary number of alleles and loci with additive, dominance and additive × additive gene actions. The mean relative response to selection increased markedly with the number of generations (0.68, 0.83, 0.92, 0.96 and 0.98 for 1, 2, 3, 4 and 5 generations, respectively) of selfing. Thus selection in a SSD population may be initiated in F_5 or F_6 ; little further gain is expected by delaying it further. The type of gene action would have some effect on the

selection response but this effect would be greatly reduced with the increased number of generations of selfing.

In a breeding programme, however, the breeder is more concerned with the proportion of homozygous lines that are superior to the better parent involved in the cross than with the mean and variance of all the possible lines that can be extracted from the cross. Jinks and Pooni (1976) developed a simple procedure for estimating the probability of obtaining inbred lines that fall outside the parental range or exceed the F_1, if it shows heterosis, by a specified amount. In the case of simple additive, dominance and additive environmental model, the probability of an inbred line having a higher score than P_1 (the superior parent of the cross) or a lower score than P_2 (the inferior parent) will be approximated by the two-tail normal probability integral corresponding with the value given by the following formula provided the distribution of inbreds derived from the cross is approximately normal.

$$= \frac{(d)}{\sqrt{(D+E)}}$$

where, (d) is the additive gene effect, D is the additive genetic variance and E is the additive environmental variance. In practice, E may be ignored since a breeder would be interested in the average performance of inbreds over environments.

In case the F_1 shows heterosis, the probability of obtaining inbreds superior to the F_1 will be approximated by the one-tail normal probability integral corresponding with the value obtained from the following formula.

$$= \frac{(h)}{\sqrt{(D+E)}}$$

where (h) is the dominance gene effect.

In the presence of additive × additive epistasis (i), the estimates of probabilities become biased. If i is positive, the probability of a derived inbred exceeding the superior parent P_1 will be less than that of a derived inbred being inferior to P_2 ; the reverse is the case when i is negative. If the distribution of inbreds is normal, the probability of obtaining inbreds that score higher than P_1 will be the one-tail normal probability integral corresponding with the value yielded by the following formula.

$$= \frac{(d) + (i)}{\sqrt{D+I}}$$

Similarly, the probability for inbreds scoring lower than P_2 will be the one-tail probability integral corresponding to the value obtained from the following formula.

$$= \frac{-(d) + (i)}{\sqrt{(D+I)}}$$

where, *i* is the additive × additive epistasis gene effect and *I* is the additive×additive genetic variance. In general, we will be unable to obtain a precise estimate of $D+I$ from the earlier generations of crosses, but in such cases, the estimates of *D* itself would be biased upward due to a significant *i*. The sum of the above two probabilities yields the probability of inbreds falling outside the parental range ; this probability will be underestimated if an allowance for significant *i* is not made.

These probability estimates are affected by linkage. A predominantly repulsion phase linkage would tend to underestimate the probability, while a predominantly coupling phase linkage would tend to overestimate it. The presence of linkage and its predominant phase can be detected by comparing the variances of F_2 and $F_2 \times F_1$ generations from a triple test cross analysis. The presence of genotype × environment interaction is not expected to present a problem as long as the interaction is a linear expansion or contraction of scale, *i.e.*, the phenotypic differences between genotypes are magnified in one environment relative to the other with little or no change in ranking. In practice, this seems to be the most likely form the integrations would take unless the environments are particularly diverse (Jinks and Pooni, 1976).

Subsequently, Pooni and Jinks (1978) extended the method for predicting the probability of an inbred exceeding the P_1 with respect to two, three or more characters simultaneously. The parameters required for these predictions can be obtained from the six basic generations, *viz.*, P_1, P_2, F_1, B_1, B_2, and F_2 and a triple test cross. The predicted proportions of inbreds exceeding the parental range for a single character (Jinks and Pooni, 1976) as well as those for two or three characters considered together (Pooni and Jinks, 1978) showed a good agreement with the values obtained from the inbreds derived through SSD from simple intervarietal crosses in *Nicotiana rustica*. Further, there was a close agreement between the predicted and observed proportions even when the distribution of the inbreds derived from the crosses was significantly nonnormal (Pooni *et al.*, 1977).

Pooni and Jinks (1979) considered the various sources of bias in the parameters used for the prediction on the properties of the inbreds produced from a cross by SSD. They used the data from *N. rustica* crosses for testing the validity of their predictions. They concluded that the estimate of *D* could introduce appreciable bias in the predictions if epistatic gene effects were significant. In the absence of epistasis, estimates of *D* from F_2 and the two backcross (B_1 and B_2) generations were adequate. But in the presence of epistasis, *D* estimates obtained from F_3 families appeard to be the best, while those from F_2 and backcrosses were the worst.

Experimental Results. Homozygous lines derived through SSD generally exceed the parental limits for various traits, indicating that transgressive segregants are retained during the procedure. For

example, in safflower 64 of the 379 BC_2F_4 lines derived through SSD from the cross (SH 202 × Oleic Lead) × SH 202 outyielded the higher yielding parent SH 202, 95 lines had higher oil content than Oleic Leed (the superior parent for the trait), and 62 exceeded the superior parent in terms of yield × oil content (Fernandez-Martinez and Dominquez-Jimenez, 1981).

There is generally some plant loss when a population is handled according to SSD, which may affect gene and genotype frequencies in such populations. Martin *et al.* (1978) investigated the effects of population density on gene frequencies for some qualitative and quantitative characters in three crosses of soybean handled according to SSD for four generations. At the low population density (Ca. 170 plants/m^2), gene frequencies for qualitative characters (flower colour and pubescence colour) did not deviate from the expected ones, while at the high population density (Ca. 510 plants/m^2) there was a significant deviation in most of the cases. This significant deviation was attributed to the high plant loss at the higher population density. The means for certain quantitative characters were also affected by population density : progenies from the high density SSD population tended to be slightly later in maturity (in 3 crosses), were slightly taller (in two crosses), had slightly higher lodging score (in two crosses), and had slightly bigger seeds (in one cross) than those from the SSD populations grown at low density. Generally, these differences in the population means were relatively small in magnitude. However, the among line variances for the four quantitative traits did not differ significantly between low and high density SSD populations of the three crosses, except for seed size in one cross where the variance from the low density population was significantly higher.

Plant Loss. In each generation, some seeds would fail to germinate, some plants would die due to insect and disease attacks, while some plants may fail to flower and set seed. Consequently, a proportion of plants may be lost in each generation and the population size may progressively decline with the advancing generation due to the limitation (only one seed from each surviving plant composited to obtain the next generation) set by the SSD procedure. Results from several studies indicate that SSD population size declines at rates ranging from 4.75 to 30.3 per cent of the population size in each generation (Table 17.2). The magnitude of plant loss in these studies appears to vary depending upon the crop species and the conditions of the experiment. Mungbean appears to be more prone to plant loss than other crop speciess probably due to its high susceptibility to diseases and environmental stresses. Similarly, populations maintained at a higher population density may suffer a greater plant loss than those cultivated at lower plant densities (Martin *et al.*, 1978).

Thus plant loss appears to be a serious weakness of SSD scheme. In situations of relatively high plant loss, gene and genotype, frequencies may be altered (Martin *et al.*, 1978) so that the

Table 17.2. 'Plant loss in the segregating populations of some crops handled according to SSD scheme.

Crop	*Population density (plants/m²)*	*Plant loss (per cent/generation)*	*Generations advanced in*	*Reference*
Lentil	—	6.2 (4.0—9.7)*	Greenhouse	Haddad and Muehlbauer (1981)
Mungbean	Ca. 130	30.3 (25.7—36.6)	Field (two crops per year during spring and kharif)	Singh *et al.* (in press)
	Ca. 200	28.3 (17.8—43.9)		
	Ca. 330	25.2 (16.1—31.2)		
Soybean	—	Ca. 15**	Greenhouse	Martin *et al.* (1978)
	Ca. 170	4.75 (3.75—6.5)	Greenhouse	-do-
	Ca. 510	13.75 (11.75—15.5)	Greenhouse	-do-
Safflower	Ca. 256	5.0	Growth Chamber	Fernandez-Martinez and Dominguez-Jimenez (1981)
Wheat	Ca. 1220	<10.0	Greenhouse (low nutrient, continuous light)	Tee and Qualset (1975)

*Values within parenthesis represent the range of plant loss in different crosses in the same study.
**Approximate plant loss estimated from several breeding populations.

genotypes retained in an SSD population may not be a random sample from the population. At least two approaches have been suggested for preventing plant loss. *Firstly*, 2-4 seeds from each plant of the previous generation may be planted in a single hill and thinned to one plant/hill at the time of flowering. This scheme involves considerable labour in harvesting, maintaining and planting the seeds of each plant separately and later in careful thinning. Further, in some situations it may fail to achieve the desired result (Singh *et al.*, in press).

Alternatively, two seeds from each plant or all the seeds from one pod of each plant (Byth *et al.*, 1979) may be bulked to raise the next generation. This modification amounts to a restricted bulking minus the effects of natural selection. In the early generations, populations obtained through such a scheme may not be markedly different in their composition from those derived through the conventional SSD scheme. However, as the homozygosity increases, such populations may become closer to bulk than SSD populations. Such a modification of the SSD scheme may, therefore, fail to prevent the shifts in gene and genotype frequencies due to random drift from plant loss and due to sampling error, although it may be able to maintain the population size.

The plant loss, therefore, may lead to an erosion in genetic variability of SSD populations. However, the results reported so far (Martin *et al.*, 1978 ; Singh *et al.*, in press), do not support this expectation.

PEDIGREE Vs. SSD

Casali and Tigchelaar (1975a, b) compared PS, SSD and BP (bulk procedure) through computer simulation studies. They found that for characters with high heritability, PS was the most effective, while SSD was the most effective for traits with low heritabilities. Further, SSD was effective even for traits having very low heritability (10%). SSD also offered the greatest benefits in situations where simultaneous selection for several characters having different heritabilities has to be done (this essentially is the situation in almost all breeding programmes).

A comparison between the effectiveness of PS and SSD methods is afforded by the results from several studies. In general, the lines derived through SSD are comparable, in some cases superior, to those developed through PS. Knott and Kumar (1975) selected 300 plants from among 2,500 F_2 plants of each of the two wheat crosses, *viz.*, Wisconsin 261 × Pitic 62 and Wisconsin 261 × Manitou. The F_2 plants were selected for resistance to rusts and root rot, straw strength, height, head type, maturity and seed type. The same selected F_2 plants were handled according to SSD as well as PS. The SSD procedure was followed in F_2, F_3 and F_4 to obtain F_5 lines ; some F_5 lines with obvious weaknesses were eliminated

and the remaining were yield-tested in F_6. The 300 F_3 lines from each of the two crosses were also grown in separate replicated yield trials ; the F_4 progenies were derived from the selected F_3 lines (on the basis of yield test) and advanced in an off-season nursery to F_5. The F_6 SSD and F_5 PS lines from a cross were planted in a replicated yield trial ; separate trials were planted for each cross. The mean yields of PS lines were significantly higher than those of the SSD lines in both the crosses mainly because there were fewer very poor yielding PS lines (Table 17.3). Correlations between the F_3 and F_5 yields of PS lines were significant in both the crosses but they were relatively low (0.29 and 0.14). A comparison between the yields of F_5 PS lines derived from the highest yielding 20% of F_3 lines and the highest yielding 20% of the SSD lines revealed that in each cross the SSD lines were at least as good as the PS lines (Table 17.3).

Boerma and Cooper (1975) extracted F_8 lines from four soybean crosses according to SSD or PS, the latter method was either based on visual selection or on early testing. The SSD lines were yield-tested in F_7 and a variable number of the highest yielding lines were included in the F_8 yield tests. Mean yields of all the selected lines, the mean yields of the five highest yielding lines, and the yield of the highest yielding line from each population showed no consistent differences between the procedures of selection (SSD and PS). The lines from early testing procedure were consistently later in maturity than those from SSD and visual selection. The authors concluded that SSD was the most efficient since it required less selection effort, avoided expensive yield testing until later generations, and allowed a more rapid generation advance.

Jinks *et al.* (1977) compared the performance of a random sample of 59 F_7 lines developed through SSD from a cross (V2 × V12) of *N. rustica* with that of lines selected according to PS for high and low mean performance in combination with high and low sensitivity to a macroenvironment variable (planting dates) for two morphological traits (flowering date and final plant height). Evaluations of these lines were made in eight different environments. In all the environments, few to several SSD lines were better than the PS lines (Table 17.4). These results demonstrate the superiority of SSD over PS for the isolation of superior recombinant lines. The inefficiency of PS was attributed to seasonal differences. Further, it was concluded that mean performance and environmental sensitivity were largely under the control of different genes.

The effectiveness of early testing in F_2 and SSD procedures in two cowpea (*Vigna unguiculata*) crosses was compared by Ntare *et al.* (1984). Both early testing and SSD populations were initiated from the same sclected F_2 plants in each cross. The F_5 SSD and early testing lines were compared in yield tests. There was significant correlation (r=0.51 to 0.85) between the yields of F_3 parental and F_6 progeny lines. Further, there was significant correlation

Table 17.3. Mean yield (expressed as per cent of checks) of F_5 pedigree lines and F_6 SSD lines derived from two crosses of wheat (from Knott and Kumar, 1975)

Breeding method	*All the Lines*			*Highest yielding 20% of the lines*		
	Number of lines	*Mean yield*	*Range*	*Number of lines*	*Mean yield*	*Range*
			Wisconsin 261 × Manitou			
SSD	232	81.9	39.2—117.8	46	98.5	91.5—117.8
Pedigree	180	85.8	48.0—115.5	36	92.7	80.6—114.9
			Wisconsin 261 × Pitic 62			
SSD	190	91.8	52.3—126.4	38	109.5	103.0—126.4
Pedigree	222	103.6	69.1—131.7	44*	108.3	75.8—131.2

* The F_5 lines were derived from the 20% highest yielding F_3 lines.

Table 17.4 The distribution of 59 SSD lines derived from the cross $V_8 \times V_{12}$ of *N. rustica* for flowering time and final height in conjunction with high and low sensitivity to environment, and the number of SSD lines superior to the two selected lines for each charcter environmental sensitivity combination (based on Jinks *et al.*, 1977).

Mean performance of the character	*Environmental sensitivity*	*Number of SSD lines*	*Number of SSD lines superior to the two appropriate pedigree selection lines*
		Flowering time	
High	High	11	7
High	Low	12	12
Low	High	20	20
Low	Low	16	1
		Final plant height	
High	High	21	5
High	Low	9	9
Low	High	13	13
Low	Low	16	1

between visual ratings of F_3 and F_6 lines for yielding ability and their actual yields indicating that it is possible to visually identify high yielding cowpea lines. However, the grain yields of SSD and early testing lines were comparable, indicating a lack of superiority of the early testing procedure. Similarly, there was no difference between SSD and PS lines of *N. rustica* with respect to seven quantitative characters. These lines were extracted from a heterotic cross. Further, the data on these lines confirmed that inbred lines which outperform the heterotic F_1 can be readily extracted from it by either SSD or PS method (Jinks and Pooni, 1984).

Dahiya *et al.* (1986) compared the performance of mungbean (*V. radiata*) lines extracted from a broad base population (developed by ordering 15 different purelines in complex crosses). These lines were developed through pedigree (visual selection for two generations), SSD and early testing (each for two generations). SSD appeared to be the most effective as more SSD lines outyielded the best check. The mean yields of all SSD lines, 10% top-yielding SSD lines and the highest yielding SSD line were the highest. The early testing procedure was found to be the least effective. Although the differences in mean yields of SSD and PS lines were consistent, their magnitude was rather small.

These data do not indicate any marked advantage due to the pedigree method (with or without early testing) over SSD. This is mainly because the selection for yield and several other quantitative traits is relatively ineffective in the early segregating generations.

Therefore, SSD procedure seems preferable to PS since the latter involves selection effort, maintenance of pedigree records, expensive yield trials for early testing, and imposes a limit on the lines that can be handled during the segregating generations. In the SSD procedure, on the other hand, there is little, if any, selection effort and yield test expenditure, no limitation on population size, no evidence for any loss in genetic variability, and an opportunity for a very rapid advance of generations (upto 4-5 generations/year with a good green-house facility). Further, a selection for highly heritable agronomic characters may be practised in F_2 while initiating the SSD population from a cross (Knott and Kumar, 1975). However, such a selection may result in the rejection of high-yielding lines before they are recognised as such. This may partially explain the relative ineffectiveness of PS. The lack of selection in SSD, except perhaps in F_2, allows the production of character combinations through recombination which might not have been selected for in PS ; such recombinants may not be of high agronomic value but may nevertheless be high yielding and of potential value as breeding stocks (Snape and Riggs, 1975).

DOUBLED HAPLOID TECHNIQUE

The doubled haploid (DH) technique of obtaining homozygous lines of self-fertilized crops is becoming increasingly popular, particularly in rice, wheat and barley, as it represents a saving of 4-5 years. Theoretically, the means and variances for a quantitative trait will be similar for the populations of SSD and DH lines irrespective of the genetic architecture of the trait, provided there is (1) no linkage and (2) no selection of gametes or genotypes in either population (Snape, 1976). In addition, the frequency of each homozygous genotype is expected to be $(\frac{1}{2})^k$, where k is the number of segregating loci.

But the means and variances of the SSD and DH populations would differ in the presence of linkage ; the direction and the magnitude of these differences would depend on the initial linkage phase, the recombination frequency and the presence of epistasis. In the cases of coupling phase linkage with complementary epistasis or repulsion phase linkage with duplicate epistasis, the mean of DH population will be higher than that of SSD population, while the reverse will be the case when coupling phase linkage is associated with duplicate epistasis or repulsion phase linkage is present with complementary epistasis. Variances of SSD populations will be greater than those of DH populations if the positive alleles are initially present in the repulsion phase, but the opposite will be true if they are initially in the coupling phase. Epistasis will tend to increase this difference in the case of repulsion phase, but it will reduce the difference when alleles are present in coupling phase. Since in most plant breeding situations repulsion phase linkage may be expected to predominate, SSD may be expected to be superior to the DH technique in releasing the maximum variation from a cross. This is mainly because recombination between the linked genes is limited

to F_8 in the case of DH, while in SSD it continues to occur till later generations, albeit in progressively decreasing frequencies. The superiority of SSD procedure may be expected to be the maximum at intermediate recombination frequencies (p=0.293 for coupling, and p=0.17 for repulsion ; p=recombination frequency ; Snape, 1976).

DH lines of tobacco developed through anther culture followed by midrib culture of haploid plants are genetically stable and do not show detectable cytogenetic anomalies (see Schnell *et al.* 1980). Similar results have been reported for barley DH lines derived through interspecific hybridization with *Hordeum bulbosum* (see Park *et. al.*, 1976). DH lines developed from plants heterozygous for qualitative traits showed typical Mendelian segregation ratios in some studies, while in other reports evidence for a preferential development of some gametes into plants were obtained. In addition, DH populations derived through anther culture from highly inbred parents show variability for quantitative traits as well as reduced vigour. This variation has been attributed to : (1) residual heterozygosity in inbred parents, (2) mutagenic effects of colchicing (used for chromosome doubling) and (3) mutagenicity of the anther culture technique itself (see, Schnell, *et al.*, 1980). However, studies with cotton and barley indicate that there may be no disadvantage associated with complete homozygosity of DH lines, and that their yield stability is not affected (see Park *et al.*, 1976).

Schnell *et al.* (1980) obtained 50 DH lines through anther culture and 50 random F_8 lines through SSD, from a single F_1 plant from the cross Hicks Broad Leaf × Coker 139 of *N. tabacum*. The performance of DH and SSD lines, alongwith suitable checks, was evaluated in three environments. The DH population yielded, on an average, 12.6% less cured leaf, had inferior leaf quality, was later in flowering, produced reduced leaf numbers and its cured leaf was lower in total alkaloids than the SSD population. The DH population showed a greater genetic variation for leaf yields that the SSD population, but the lower mean in the case of former negated the benefit of increased variation. For example, none of the DH lines was even equal to Coker 139, the best check, for leaf yield, while one SSD line outyielded this check. Although the predicted gain in leaf yield from selection in the DH population (7.8%) was larger than that in SSD (4.5%), the selected SSD lines were expected to be markedly superior (2,436 kg leaf/ha) to the selected DH lines (2,246 kg leaf/ha). It was, therefore, concluded that selection in SSD populations would be preferable to that in DH population for the identification of superior genotypes. The possible causes for the inferiority of the DH population were suggested to be (1) incomplete inbreeding in the SSD population, (2) repulsion phase linkage, (3) mutagenic effects of colchicine treatment, (4) mutagenicity of the anther culture procedure, and (5) differential development of microspore genotypes into embryoids.

Park *et al.* (1976) developed 100 lines through each of the three procedures, *viz.*, SSD (F_6), pedigree (visual selection, F_6) and DH (haploids obtained through interspecific hybridization with *Hordeum bulbosum*), from two barley (*H. vulgare*) crosses. From these lines, 52 random lines for each procedure in each cross were included in yield trials at two locations over a period of two years. There was no difference for grain yield, heading date and plant height between the DH lines and those derived through SSD and PS in terms of means, variances and the frequencies of desirable genotypes (Table 17.5). Further, there was no evidence for deleterious effect of complete homozygosity in the DH lines ; the DH lines were as good agronomically as the SSD and PS lines. It was concluded that the DH technique is a very useful tool for producing high yielding homozygous barley lines.

Choo *et al.* (1982) developed DH (through the *H. bulbosum* technique) and SSD lines from two barley crosses. The frequency distributions of DH and SSD lines for grain yield, heading date and plant height were compared by Mann-Whitney U test, Kolmogorov-Smirnov two-sample test and Wald-Wolfowitz runs test. The distributions of DH and SSD lines for the three metric traits were comparable. Although the SSD procedure allows a greater opportunity for recombination than the DH method, it did not produce a sample of recombinants which differed significantly from those derived through the DH technique.

The performance of barley lines developed through PS and DH (anther culture) techniques was compared by Feidt and Foroughi-Wehr (1986). Five PS and 5 DH lines were extracted from each of the five barley crosses ; they were planted in a replicated yield trial in different environments (represented by locations and years). The performance of PS and DH lines was comparable in three of the five crosses. In one of the remaining two crosses, two DH lines were significantly lower yielding than PS lines, while in the other cross two DH lines were significantly higher yielding than PS lines. Clearly, the DH lines represent a nonrandom sample from the five barley crosses, indicating that there is a considerable unconscious selection in the desirable direction during their isolation through anther culture.

In cereals like wheat and barley, two techniques are available for the production of DH lines : (1) anther culture and (2) interspecific hybridization with *H. bulbosum*. The relative merit of these techniques has been examined in some detail by Snape *et al.* (1986). They have proposed three criteria for the comparison of DH production techniques : (1) easy and consistent production of large numbers of doubled haploids of all the genotypes in the breeding programme, (2) production of normal and genetically stable DH lines, and (3) absence of selection during the production of DH lines. Both anther culture and *H. bulbosum* techniques are quite successful with some genotypes of barley and wheat, but not with others.

Table 17.5. Mean and range for yield (over two locations and two years) of F_5 SSD, F_6 pedigree and DH barley lines derived from two crosses ; the data pertain to 52 lines for each method in each cross and to 30% of the top-yielding lines for each procedure (from Park *et al.*, 1976).

Breeding method	*Yield of all the lines (g/hill)*		*Yield of top 30% lines (g/hill)*		
	Mean	*Range*	*Number of lines*	*Mean*	*Range*
			Paragua × Zephyr		
Pedigree	18.1[a]**	13.5—30.8	7**	22.6	19.2—30.8
SSD	18.8[a]	7.6—27.3	16	24.4	21.1—27.3
DH	17.2[a]	7.6—26.2	16	22.9	19.9—26.2
			OB 7358 × Champlani		
Pedigree	20.4[a]	11.8—37.5	16	26.4	22.4—37.5
SSD	21.5[a]	7.5—35.1	16	28.1	23.7—35.1
DH	22.1[a]	9.5—34.4	16	28.6	24.7—34.4

* Values followed by the same letter (within each cross) are not significantly different from each other.

** Selected only 7 lines out of 21 lines for the top 30%.

However, a steady progress is being made in improving the efficiency of these techniques. At present, the *H. bulbosum* technique appears to be markedly more efficient in producing green haploid plants than the anther culture technique both in wheat and, particularly, in barley (Table 17.6). Both the systems require comparable facilities and financial commitments. But in terms of man-hours required for the production of every DH line, anther culture (0.3 man-hours/DH line) appears to be relatively more efficient than the *H. bulbosum* technique (0.5 man-hours/DH line).

Table 17.6. The relative effectiveness of anther culture and *H. bulbosum* techniques in generating haploid lines of wheat and barley (after Snape *et al.*, 1986)

Method	*Anthers cultured or florets pollinated*	*Albino plants or seeds set**	*Green plants*	*Green plants per 100 anthers or per 100 florets***
		Wheat		
Anther culture	28,000	—	890	3,2
H. bulbosum system	1,020	—	55	3.4
		Barley		
Anther culture	7,020	54	10	0.14
H. bulbosum system	1,908	1654	218	11.40

*Albino plants in the case of anther culture, and seeds set in the case of *H. bulbosum* system.

**Per 100 anthers in the case of anther culture system, and per 100 florets in the case of *H. bulbosum* system.

There is a considerable evidence to show that both the techniques induce genetic variation in addition to that generated by segregation and recombination in the F_1 hybrids. In addition, these techniques appear to impose a selection pressure so that the lines derived through them are essentially nonrandom. Snape *et al.* (1986) feel that critical studies are required to assess the usefulness of such variations and of selection pressures in breeding programmes. A comparison of spring barley DH lines derived from two crosses through the anther culture and the *H. bulbosum* techniques showed that the latter had significantly superior yield/plant (in both the crosses) and 100-grain weight (in one cross) than the former, while both the groups had comparable grain number/spike. However, more data are required from other crosses in barley and from those in wheat to allow a meaningful generalisation.

Thus, DH lines are either comparable or inferior to SSD lines in yield and other quantitative traits, but in some cases, they may be comparable to PS lines. The development of DH lines either through

anther culture or through interspecific hybridization (for embryo rescue) requires dependable tissue culture and greenhouse facilities. On the other hand, a rapid isolation of SSD lines requires only a dependable greenhouse facility. It is doubtful that the saving of one year through DH procedure over SSD (assuming 3-4 generations per year with the help of a greenhouse) would justify the effort and expenditure involved in the production of DH lines (particularly in the Indian context). In most developing countries, however, only few plant breeders would have an access to tissue culture and greenhouse facilities. Often, these facilities may not be wholly dependable leading to frequent losses of valuable breeding materials. Consequently, breeders of developing countries would generally have to depend on off-season nurseries for advancing the generation of their materials ; in such a situation, SSD appears to be the only feasible procedure for a rapid isolation of homozygous lines.

BULK METHOD

When breeders handle segregating generations from crosses of self-pollinated crops according to the bulk scheme, they have some explicit or implicit expectations which may be summarised as follows. In *long-term bulks*, natural selection is expected to (1) improve the mean performance of bulk populations, and (2) to retain agronomically superior genotypes in the population, or even (3) cause an increase in their frequency thus facilitating their isolation in later generations. (4) In *short-term bulks*, on the other hand, it is hoped that there would be no shift in gene and genotype frequencies due to either natural selection or random drift. Thus in the case of long-term bulks, the gene and genotype frequencies of the population are expected to change for the better, while short-term bulk populations are hoped to contain a random sample from the all possible homozygous genotypes obtainable from the initial population.

The effectiveness of natural selection has been studied in varietal mixtures and in both long and short-term bulks. In addition, many workers have combined mass selection with the bulk procedure (BP) to achieve specific goals.

Varietal Mixtures. Data on changes in frequencies of different pure lines are available from studies on varietal mixtures of several crops, *e.g.*, barley (*H. vulgare*), wheat (*T. aestivum*), soybean (*G. max*), rice (*O. sativa*), rajma (*P. vulgaris*) etc. In general, the frequencies of different varieties constituting a mixture undergo marked changes within few generations, and one or two genotypes tend to become predominant with time. Further, different varieties may become predominant at different locations or under different environments (see Allard, 1960). The ability of a genotype to survive in mixture is generally referred to as *competitive ability* or *fitness*.

Evidently, different varieties of various crops differ in competitive ability. But the crucial question is whether competitive

ability is positively associated with yielding ability and/or agronomic desirability. Theoretically, if yield and fitness are positively associated, superior yielding genotypes would tend to become predominant. Consequently, BP would be more efficient than PS and SSD procedures. Further, genotypes having high seed set and lower seed weight (or seed size) may be expected to be more competitive than bolder seeded genotypes ; this appears to be the case in many crops (*e.g.*, Khalifa and Qualset, 1974, in wheat ; Hamblin, 1975, in lentils ; Roy, 1976, in wheat). However, smaller-seeded genotypes, even if higher yielding than bold-seeded ones, may not be desirable from the commercial viewpoint.

In some early studies, agronomically poor barley varieties were found to be poor competitors in varietal mixtures, but in some other investigations the competitive ability was not associated with yielding ability (see Allard, 1960). Suneson (1949) concluded that 'the bulk population method of breeding will not necessarily perpetuate the highest yielding or the most disease resistant progenies, but that otherwise intangible character of competitive ability may measure other very important characteristics'. These apprehensions have since been supported by data from several studies. In wheat (Khalifa and Qualset, 1974), rice (Jennings and DeJesus, Jr., 1968), and soybean (Mumaw and Weber, 1957) survival in varietal mixtures was not related to yieiding ability. In wheat, rice and barley, natural selection tends to favour tall, leafy and high tillering plant types, while the high yielding ideotype is short with narrow, erect leaves and fewer tillers. Results obtained from studies on the segregating generations of crosses in wheat (Khalifa and Qualset, 1975) and rice (Jennings and Herrera, 1968) support these conclusions. Similarly, small-seeded, low yielding rajma (*P.vulgaris*) genotypes tend to eliminate high yielding, large-seeded types (see Hamblin and Evans, 1976). In addition, determinate (bush) type soybean and rajma genotypes

Table 17.7 Performance of DH lines derived from two barley crosses through anther culture and *H. bulbosum* techniques (after Snape *et al.*, 1986)

Method	*Number of lines*	*Grains/ spike*	*100-grain weight (g)*	*Yield/plant (g)*
		E 1388×1506 C		
Anther culture	19	26.02	5.203	11.73
H. bulbosum	19	26.34	5.668*	13.95*
Mid-parent value	—	26.59	5.333	12.84
		Franken × Trumpf		
Anther culture	21	25.38	4.775	11.06
H. bulbosum	21	26.30	4.875	13.08*
Mid-parent value	—	25.88	4.291	11.14

*Significant difference between the means of anther culture and *H. bulbosum* derived DH lines.

are poor competitors against indeterminate (vine) types ; there is no reason why the same may not hold true for other pulse crops (Haddad and Muehlbauer, 1981).

Clearly, natural selection tends to act against certain desirable features of some crops since they appear to have poor survival value in mixtures. Obviously, it would favour only those characteristics that are positively associated with competitive ability of fitnesss.

Long-Term Bulks. Long-Term bulks (bulking for 10 or more generations) of segregating generations from crosses are maintained in the hope that frequencies of high-yielding genotypes would increase, while those of poor-yielding genotypes would decrease with time due to natural selection. Several studies have been conducted on long-term bulks (see, Jain, 1951 ; Allard and Jain, 1962 ; Choo *et al.*, 1980 ; Mac and Harvey, 1982). Most of these studies have been conducted with composite crosses of barley. A *composite cross* may be generated in one of the following three ways : (1) bulking equal quantities of seeds from F_1 or F_2 generations of surveral crosses, (2) involving several parents/lines into a complex cross, and (3) bulking a homogeneous or heterogeneous male sterile line alongwith several selected lines/varieties and harvesting the seed from male sterile plants only (Mac and Harvey, 1982).

In most of these investigations, it was found that the mean performance of bulk population increased with time. The results reported by Suneson and Stevens in 1953 (see Allard, 1960) are remarkable in that the yield of Composite Cross II (CC II) of barley increased from 67.6% of Atlas in F_3-F_4 to 135.7% of the same variety in F_{24} (Table 17.8). This represents an increase of about 68% in the yield of CC II over a period of 20 years of bulking, which amounts to an average yield increase of 3.4% per generation. This rate of yield increase compares favourably with that obtained through mass selection in cross-pollinated crops (see Chapter 8). These findings become more remarkable when one considers the performance of

Table 17.8. The effect of bulking period on the yield of Composite Cross II (CC II) of barley (After Suneson and Stevens, 1953)

Generation of Composite Cross II	*Year of evaluation*	*Yield (Bushels/acre)*		*Yield of CC II as per cent of Atlas*
		CC II	*Atlas*	
F_3—F_4	1929—30	58.3	86.2	67.6
F_7—F_8	1933—34	71.3	83.8	85.0
F_{11}—F_{14}	1937—40	72.6	81.7	88.8
F_{15}—F_{20}	1941—46	75.1	70.8	106.0
F_{21}—F_{23}	1947—49	56.8	49.9	113.8
F_{24}	1950	51.3	37.8	135.7

pure-lines isolated from the different generations of CC II. The top three selections from F_{20} of CC II outyielded Atlas by an average of 56% in four years of replicated trials ; these selections were also moderately good in agronomic characteristics. Similar encouraging results were obtained from some other long-term barley bulks (see Allard and Jain, 1962 ; Jain 1961).

In contrast, results from other studies have not been as dramatic. Allard and Jain (1962) and Jain (1961) examined in detail the materials developed by Suneson and concluded that the yield and fitness of barley Composite Cross V increased upto the eighteenth generation, the latest generation evaluated by them. Similar results have been obtained with the populations of several self-pollinated species which had been grown as bulks for many generations (see Allard *et al.*, 1968). Most of these evaluations were carried out at low density, which would have promoted profuse tillering. However, at commercial crop densities genotypes would not produce many tillers despite their high tillering capacity. In addition, high tillering capacity may not necessarily reflect high yielding potential at high crop densities. Some authors even feel that when yield per unit area is the objective, high tillering capacity may be a nuisance. It has been clearly demonstrated that data obtained at low densities are of little use in determining the yield potential of crosses at crop densities. In addition, the competitive ability of different genotypes may change with population density (see Hamblin and Evans, 1976).

Choo *et al.* (1980) grew 17 single cross bulks of barley at two locations ; comparisons were made by growing F_{10} and F_{15} generations of each of the 17 populations at the two locations for two years. They observed that natural selection favoured longer awns, shorter spikes, smaller flag leaves, and fewer spikes per plot, but it had little effect on the number of grains/spike or grain yield. The effect of location was noticeable only in F_{15} on awn length, spike length, flag leaf area and number of grains/spike.

The exploitable genetic variation present in Composite Cross XXI (ÇC XXI) of barley was estimated by Mac and Harvey (1982). CC XXI was generated by compositing seeds from 6,200 entries from USDA world collection and 7,500 male sterile plants having Coast; Manchuria, Trebi, Hanchen, CC XIV and CC XV backgrounds ; seeds from the male sterile plants were harvested in bulk in 1961 and distributed as CC XXI. Mac and Harvey isolated 284 lines from generation 4 (G4) and 307 lines from G12 of CC XXI. The mean performance of G 12 lines showed an increase in heading date, maturity date and grain yield, but a decline in kernel weight as compared to G 4 lines. However, the coefficient of variation for these characters was comparable in the two populations. They concluded that although composite crosses show slow progress, they are inexpensive to maintain

and that they can provide useful reservoirs of variation which would complement the traditional world collection.

Short-Term Bulks. Many plant breeders do not feel enthusiastic about maintaining long-term bulks simply because of the long period of time involved. Still BP may be utilized for the isolation of homozygous lines with the least expenditure and effort ; for this purpose, short-term bulks of 5-8 generations are maintained. It is, however, necessary to examine if changes in gene and genotype frequencies take place in short-term bulks. Many studies have focussed on this question, and several authors have attempted to compare the effectiveness of short-term BP with SSD and PS.

Khalifa and Qualset (1975) grew F_3 to F_6 generations of two wheat crosses as bulks under field conditions, and recorded the height of individual plants. Yield as well as mean height of the population increased, while the frequency of short-statured plants decreased with generation. Mean yields of lines extracted from F_3 F_4, F_5 and F_6 generations showed a significant increase with generation. Some of the F_7 lines (derived from F_6 bulk) were superior to both the parents in yielding ability. Evidently, there was a directional and stabilizing selection for both plant height and yield in these short-term bulks.

The effect of short-term (upto F_6) bulking in two high-yielding, and two low yielding crosses of rajma (*P. Vulgaris*) was studied by Hamblin (1977). Bulking did not alter the mean performance of the two high yielding crosses, but there was a steady increase with generation in that of the two low yielding crosses. In this study, the progeny variance of crosses did not markedly differ from the parental variance possibly due to a large environmental (interplant competition) effect on the expression of quantitative traits of this crop. It was concluded that "cross variance, for characters which are much influenced in their relative expression by the environment (of which yield is the outstanding example), has little relevance as a criterion for deciding the relative worth of different crosses at crop densities'.

These studies clearly demonstrate that shifts in gene and genotype frequencies take place even in short-term bulks, and that in most cases the performance of bulks improves with generation. However, sometimes natural selection may act against a desirable genotype, *e.g.*, short-statured plants in wheat etc. In such cases, a mass selection procedure could be effectively used to eliminate the undesirable types from the population without much labour.

Mass Selection in Bulk Populations. Taller plant stature, smaller seed size and later maturity appear to be the disturbing traits that are favoured in many bulk populations. The breeder may subject a bulk population to mass selection for shorter plants, bolder seeds and early maturity by mowing off taller heads, sieving off smaller seeds and harvesting at the desired maturity time, respectively. The effectiveness of such mass selections has been investigated by several workers.

Romero and Frey (1966) subjected a heterogenous and segregating bulk oat population to mass selection for uniform plant height. All the panicles taller than a specified height were clipped-off, and at maturity only the top 10 cm of the clipped plots was harvested. This procedure of mass selection was effective in reducing mean plant height and the genetic variance for the trait of the population. In addition, the selected population became earlier in maturity and gave higher grain yield than the original population.

Fehr and Weber (1968) applied three cycles of mass selection for seed size and specific gravity in the F_6-F_8 generations of two soybean crosses. There was an approximately linear change in the direction of selection in seed size and specific gravity with selection cycle ; there was an associated change in protein and oil contents of the seeds as well. The maximum progress for high protein and low oil was obtained by selecting for large seed size and high specific gravity. On the other hand, a selection for small seed and low specific gravity resulted in the maximum progress for high oil and low protein. Smith and Weber (1968) were able to select for protein and oil contents in soybean by mass selecting for specific gravity alone. Selection for high specific gravity resulted in a higher mean protein and lower oil content, while the reverse was the consequence of a selection for low specific gravity. The effect of continued mass selection for specific gravity varied among and within populations.

Peterson *et al.* (1986) subjected 52 bulk populations of wheat to mass selection for seed density of water-imbibed (at 0-3°C for 9-10 days) seeds ; the selection was aimed at improving seed protein content. Selection for low seed density was able to improve seed protein content in 10 of the 52 populations ; the increase in protein ranged from 6-11 g protein/kg seed. However, seed size was unaffected by selection for seed density. A large amount of nongenetic variation in seed protein content appeared to limit the effectiveness of selection for seed density.

These results indicate that mass selection for highly heritable traits can be effectively practised in bulk populations. Mass selection techniques may also be designed to eliminate highly competitive but agronomically poor types from bulk populations. The phenomenon of correlated response to selection may also be exploited. whenever such opportunities exist, to select for a relatively difficult or time-taking-to-estimate trait indirectly by mass selecting for a highly correlated more simply estimated trait, *e.g.*, selection for protein and oil contents in soybean through mass selection for seed size and seed density.

BULK Vs. PS AND SSD

Several workers have compared the effects of short-term bulking with SSD or pedigree methods on the segregating generations

from crosses of self-pollinated crops. The main objective of such studies is usually to determine if the inexpensive procedure of bulking compares favourably with the more demanding SSD, or with PS, the most demanding of the three.

Muehlbauer *et al.* (1981) used computer simulation to compare the genetic variation retained after four generations of inbreeding in BP and SSD populations of a crop with seven chromosomes, each chromosome having six loci. In F_6, the additive genetic variance in the BP population was smaller than that in the SSD possibly due to the losses in genetic variability during generation advance according to BP. Within the bulk population a considerable proportion of the total additive genetic variance originated from within line variance as opposed to that which originated from between line variance. This difference between within-line and between-line variances was considerable when the inbreeding coefficient was greater than 0.9. In the SSD population, on the other hand, all the additive variances must originate from between line variance. When the standard deviation of fecundity was greater than 25 seeds/plant, progeny from 75% of the original F_2 plants were eliminated after four generations of BP. About the same number of lines were lost after four generations of SSD when plant survival was lower than 70% in each generation of SSD. Linkage had little effect on additive genetic variance in either system, unless the two parents of the original cross had considerably different sets of alleles.

The effectiveness of BP and PS methods has been compared by many workers, and often conflicting results have been reported. In most studies, *e.g.*, those with barley and soybean, PS and BP were found to have comparable effectiveness. But in some studies, PS was reported to be more effective than BP in the isolation of high yielding lines (see Boerma and Cooper, 1975). The findings of some of the more recent studies are summarised below.

Empig and Fehr (1971) developed four populations, *viz.*, SSD, restricted cross bulk, maturity group bulk and cross bulk, from F_2 of three soybean crosses and carried them to F_5 according to the respective procedures. In F_6, 45 random progenies derived from each of the four populations of the three crosses were evaluated at two locations. Method means did not differ significantly at either location, and differences in genotypic variance among methods were not consistent in all the crosses. SSD, restricted cross bulk and maturity group bulk maintained a similar number of high yielding lines, which was about twice as many as cross bulk. SSD and maturity group bulk were the most effective in maintaining early lines. SSD was about twice as effective in maintaining large-seeded lines as other methods, while restricted cross bulk had the highest frequency of tall plants. Maturity group bulk was the most time-consuming at each harvest, followed by restricted cross bulk, SSD, and cross bulk in that order. They concluded that SSD was the "least influenced by natural selection ; therefore, it may be the most useful method in greenhouse or winter nursery environments where

a genotype may perform differently than under field conditions in its area of adaptation".

Tee and Qualset (1975) subjected two wheat hybrid populations (F_2 onward) to a rapid generation advance according to BP and SSD under low nutrient supply in a greenhouse. Random SSD and BP-derived lines were evaluated in F_4, F_5 and F_6 at two locations. Generation means showed increased plant height and yield from F_3 to F_6 in one hybrid but no change in the other hybrid with BP. But the means did not change with SSD, except for plant height in the cross where recessive genes for dwarfness were segregating. Genetic variation among lines was greater for SSD than BP in one cross, while the other cross showed the opposite picture. Mass selection for increased and decreased plant height during the accelerated generation advance was effective ; it was more effective for decreased plant height. Selection response for earliness, however, was only slight. Thus the SSD and BP populations were generally comparable except for the competition effect of plant height, *e g.*, tall plants increased in BP. It was concluded that "in a system of rapid-generation turnover where only a few seeds per plant are produced, the BP method can be applied more efficiently than SSD '. However, when the competition effects are important, SSD is preferable to BP.

Haddad and Muehlbauer (1981) advanced three lentil crosses from F_2-F_4 according to SSD and BP. In F_4, 50 random plants were selected from each population, their seeds were increased in F_5 and a replicated yield trial was conducted in F_6. SSD maintained more genetic variation than BP in 15 of the 21 comparisons of characters, *e.g.*, for days to maturity and yield in cross 1 ; height of the lowest pod in cross 2 etc. SSD-derived populations had about 11% more erect lines than BP populations, while BP maintnined a marginally higher percentage of taller types than SSD. This indicated a reduced frequency of short plants with low flowers as a result of natural selection operating within BP against less competitive short types. The authors concluded that SSD is an efficient cost-saving method of advancing lentil populations.

Rahman and Bahl (1985) advanced six hybrid populations of chickpea from F_2 to F_4 according to SSD, bulking with mass selection (MS) and random bulk (BP). In all populations, except one, means for seed yield and yield component traits were higher in MS than in SSD or BP ; the last two were more or less comparable with each other. The estimates of genetic variances appeared to follow a similar trend. It was concluded that MS was superior to SSD and BP.

Pawar *et al.* (1986) handled F_2 and F_3 generations of two wheat crosses according to BP, SSD and PS ; the performance of these populations was compared in yield tests in F_3 and F_4. The mean performance of F_3 and F_4 PS populations was superior for grain yield/plant and other yield traits than that of BP and SSD populations. Significant phenotypic coorelations between the

performances of F_2 parents and their F_3 progenies, and between the performances of F_3 parents and their F_4 progenies were observed for almost all the characters in both the crosses. The SSD procedure appeared to be superior to BP.

Segregating generations ($F_2 - F_4$) from five mungbean (*Vigna radiata*) crosses were handled according to BP and SSD; SSD populations were maintained at three different population densities. Two crops per year were grown in kharif and spring seasons. The SSD and BP populations from each cross were evaluated in replicated trials in F_3, F_4 and F_5 generations for yield and several yield traits. Mean performance of the BP and SSD populations did not differ significantly, except in a few cases, which were most likely due to sampling/experimental errors. Estimates of coefficient of variation, heritability and genetic advance were variable, but no consistent differences between the methods (BP and SSD) could be detected (Singh, 1987 ; Singh *et al.*, in press). A peculiar feature of this study was the large environmental effect on the expression of yield and yield traits of the various populations ; this is comparable to the findings of Hamblin (1977) with rajma.

CONCLUSION

Any breeding scheme for handling the segregating generations from crosses in self-pollinated crops should ensure the following : (1) an accelerated generation cycle, (2) an absence of intergenotypic competition, (3) a low mortality during generation advance, (4) maintenance of a large population size, (5) use of environments where natural selection is minimised, (6) a low labour input requirement (Tee and Qualset, 1975) and (7) a low land requirement (if off season nurseries/greenhouses are to be used). Experimental findings reveal that PS is generally not superior to SSD ; in some cases it may even be inferior to SSD. Early testing may marginally improve the effectiveness of PS over SSD, but it is doubtful if the gains justify the labour and financial commitments in early testing. Similarly, DH (doubled-haploid) technique may represent some gain over SSD in terms of time, but again the labour and fund requirements are far too high for DH than those for SSD.

In short-term bulks, natural selection tends to favour taller, smaller-seeded and often later-maturing types in several crops. But when segregation for these and other characters associated with fitness is not taking place, short-term bulk populations are comparable to those derived through SSD. Even where segregation for such traits is taking place, the bulk population may be effectively subjected to mass selection in favour of the agronomically desirable but less competitive traits. Alternatively, separate bulks, *e.g.*, tall and short-statured bulks in cereals, large and small-seeded bulks in *Phaseolus* etc., may be created for such traits to avoid the effects of natural selection. Bulks may also be subjected to rapid generation advance in a greenhouse in the same manner as SSD provided an environment that minimises the effects of natural selection is

available (Tee and Qualset. 1975). Thus in a noncompetitive situation, BP is preferable to SSD since the latter involves the harvesting of a single seed from each surviving plant of the population, and there is always some plant loss under SSD. However, when intergenotypic competition is likely to occur, SSD is preferable to BP. Thus SSD appears to be the most dependable, most rapid and the least expensive method for obtaining homozygous lines from crosses of self-pollinated crops ; in addition, SSD is as effective a breeding method as PS or often even early testing (PS with yield testing).

These conclusions, are, however, not supported by the relative success particularly in India, of the various breeding methods (PS, BP, SSD, DH) in developing high yielding varieties of various crops. In this respect, PS is by far the most successful ; in fact, not a single variety developed through BP, SSD or DH has been released in India. But the singular success of PS in varietal development and its extreme popularity with the plant breeders do not necessarily demonstrate its superiority over the other breeding methods. In fact, its extreme popularity may be the reason for its singular success in this country and *vice versa*. Few breeders in India use a procedure other than PS for handling the segregating generations from crosses of self-pollinated crops, and this may account for the lack of success of other breeding methods in the development of varieties.

Based on the experimental findings discussed in the foregoing sections, a rational breeding scheme involving the least commitment of labour, land, funds and time, and at least as effective as the pedigree method may be developed as follows. It is assumed that a dependable greenhouse facility is not available, but the breeder has an access to off-season nursery facilities.

1. Selected parents are crossed according to an appropriate mating design during the main crop season.
2. The F_1's so obtained are grown in an off season nursery to advance the generation.
3. In the following main season (second year), the F_2 populations are space-planted to facilitate visual evaluation of individual plants. Plants are selected on the basis of simply-inherited and highly heritable characters, *e.g.*, disease resistance, plant height, lodging resistance etc. Only those traits that are governed by dominant genes are selected for in F_2 ; selection for recessive traits is delayed till the F_4 generation. This is done to avoid the elimination of genotypes that may yield desirable recombinants in the subsequent generations. Seeds from all the selected plants from an F_2 population are composited. If desired, mass selection for seed size etc. may also be practised.
4. If a large number of crosses are being handled, F_2 bulks from the crosses may be planted in a replicated yield trial (in

addition to the space-planted population; item 3). Poor yielding crosses may be rejected so that a greater attention may be provided to the more promising crosses.

5. The F_2 bulks (crosswise) from selected F_2 plants are grown in an off-season nursery to advance the generation. Each F_3 population is handled according to the bulk scheme.
6. In the main season of the third year, F_4 populations are space-planted. From each population, a large number of plants are selected for simply inherited, highly heritable traits irrespective of whether they are governed by dominant or recessive genes. Seeds from all the selected plants of a population are composited.
7. The bulk F_5 populations (crosswise) are grown in an off-season nursery to advance the generation.
8. In the fourth year (main crop season), F_6 populations are space-planted, and a large number of plants having desirable characteristics are selected from each cross. Seeds from each selected plant are kept separately.
9. In the main season of the fifth year, F_7 progeny rows from the selected F_6 plants are grown in an unreplicated trial, appropriate checks are planted at regular intervals. The performance of progeny rows is compared according to either contiguous control or moving average procedure. If enough seed is available and the number of F_7 progenies is manageably small, a replicated trial may be conducted. Superior progenies are harvested as bulks for further evaluation in a replicated station trial.
10. In the sixth year, a replicated station trial is conducted ; outstanding lines (F_8) are identified for multilocation trials in the next year.

The above scheme (1) permits the breeder to exercise his skill through selections in F_2, F_4 and F_6 generations, (2) utilizes the facility of off-season nursery to save at least two years in comparison to PS (seven years for multilocation trials as against 9 years in the case of PS) and takes only three more years than DH (multilocation trials in the fourth year), (3) makes the maximum use of available resources (land, labour, funds and time), (4) avoids the record keeping of PS, and (5) permits the breeder to handle a larger number of crosses than would be possible with PS.

In case a dependable greenhouse (GH) facility is available, selection may be delayed till F_3 (F_1 and F_2 grown in GH). F_4 and F_5 are grown in GH according to SSD or BP, and F_6 onward is handled as described earlier. This represents a saving of one year as compared to the earlier scheme, and it takes only two more years than DH, the quickest procedure for obtaining homozygous lines from crosses.

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CHAPTER 18

Population Improvement

Populations of cross-pollinated crop species are highly heterozygous as well as heterogeneous. Their genetic make-up is such that they show variable inbreeding depression, which in some cases may be very severe. Consequently, breeding methods for cross-pollinated crops aim at preventing inbreeding, or at least to keep it to the minimum, in order to avoid its undesirable effects. The breeding methods commonly used in cross-pollinated crops may be grouped into two broad categories : (1) population improvement and (2) hybrid and synthetic varieties. *In the case of population improvement, mass selection or its modifications are used to increase the frequency of desirable alleles, thus improving the characteristics of the population.* In the case of *hybrid* and *synthetic* varieties, a variable number of strains are crossed to produce a hybrid population ; the strains that are crossed are selected on the basis of their combining ability.

Population improvement was the earliest breeding method applied to cross-pollinated crops. Mass selection has been used by farmers since early days and may be expected to have played a significant role in the improvement of cross-pollinated crops. The interest in population improvement methods declined with the development of hybrid and synthetic varieties. However, breeders are now paying increasingly more attention to population improvement programmes since the last two decades. The maize improvement programmes at CIMMYT, Mexico and the pearl millet improvement programme at ICRISAT, Hyderabad, are largely based on population improvement. The All India Coordinated Maize Improvement Project is placing an increasingly greater emphasis on population improvement. The population improvement methods may be grouped into two general classes : (1) without progeny testing and (2) with progeny testing.

Without Progeny Testing. Plants are selected on the basis of their phenotype, and no progeny test is carried out, *e.g.*, mass selection.

With Progeny Testing. The plants are initially selected on the basis of their phenotype, but the final selection of plants that contribute to the next generation is based on progeny test. This class of population improvement includes : progeny selection or ear-to-row method. and recurrent selection.

A more comprehensive and commonly used classification of selection schemes is presented in Table 18.1. However, the discussion in this chapter shall follow a less detailed, but equally useful and much simpler classification.

OBJECTIVES OF SELECTION

In self-pollinated crops, selection is employed to isolate plants with superior genotypes ; these plants are then used to establish separate purelines or their seeds are bulked to produce a mixture of purelines. This is possible because self-pollinated crops are naturally homozygous and generally do not show appreciable inbreeding depression. In contrast, cross-pollinated crops generally show moderate to severe inbreeding depression. Consequently, inbreeding must be avoided or kept to a minimum in cross-pollinated species. Further, individual plants from such crops are highly heterozygous ; the progeny from such plants would be heterogeneous and usually different from the parent plant due to segregation and recombination. Therefore, desirable genes can be seldom fixed through selection in cross-pollinated populations, except for qualitative traits and, perhaps, for easily observable quantitative characters with high heritability. *The breeder, therefore aims at increasing the frequency of desirable alleles in the populations.* This would result in an increase in the frequency of desirable gene combinations or genotypes. As a result, the phenotype of the population would be favourably changed. *It should be clearly understood that in cross-pollinated species the genotype of the individual plants is generally of little importance, particularly in population improvement programmes. It is the frequency of desirable genes or alleles in the population as a whole that determines the value of a population.*

MASS SELECTION

Mass selection is the oldest breeding scheme available for cross-pollinated crops. *In mass selection, a number of plants are selected on the basis of their phenotype, and the open-pollinated seed from them is bulked together to raise the next generation.* The selected plants are allowed to open-pollinate, *i.e.*, to mate at random including some degree of selfing (usually about 10% in maize, *Z. mays*). Thus mass selection is based on the maternal parent only, and there is no control on the pollen parent. Selection of plants is based on their phenotype and no progeny test is conducted. Mass selection, as applied to cross-pollinated crops, is essentially the same as that applied to self-pollinated crops. The method is outlined in Fig. 18.1. The selection cycle may be repeated one or more times to increase the frequency of favourable alleles ; such a selection scheme is generally known as *phenotypic recurrent selection.* Care should be taken to select a sufficiently large number of plants in order to keep inbreeding to a minimum. The efficiency of mass selection primarily

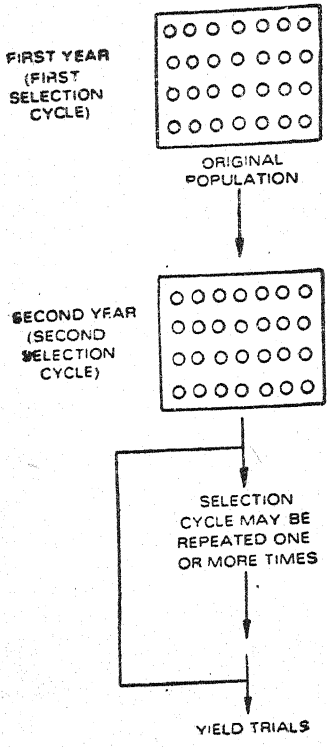


Fig. 18.1. Mass selection as applied to cross-pollinated crop species. When the selection is repeated one or more times, as outlined here, the scheme is known as phenotypic recurrent selection.

depends upon the number of genes controlling the character, gene frequencies and, more importantly, heritability.

Merits of Mass Selection

1. Mass selection is an extremely simple breeding progamme. Work of the breeder is kept to a minimum since the selections are based on phenotypes of plants.
2. The selection cycle is very short, *i.e.*, of only one generation. Thus in every generation, one cycle of selection is completed.
3. It is highly efficient in improving characters that are easily identified visually and have high heritability, *e.g.*, plant height, size of ear, date of maturity etc.
4. If proper care is taken, mass selection is effective in improving yields in cross-pollinated crops. Most cross-pollinated crops have a high additive component of genetic variance which responds to selection.

5. Since the improved strain is likely to be similar to the original population in the range of adaptation, extensive yield trials may not be required before release as a new variety.

Demerits of Mass Selection

1. Selection of plants is based on the phenotype of individual plants. Most of the quantitative characters are considerably affected by the environment. Therefore, superior phenotype is often a poor basis for the identification of superior genotype.
2. The selected plants are pollinated by both superior and inferior plants present in the population as the selected plants are allowed to open-pollinate. This reduces the effectiveness of selection.
3. High intensities of selection reduce population size and, as a result, lead to some inbreeding. Inbreeding depression may nullify the advances made under selection.

Table 18.1. A brief description of the various selection schemes for population improvement (the description is based primarily on maize).

Selection scheme	*Brief description*
INTRAPOPULATION IMPROVEMENT	For improvement within a population.
A. Mass selection	Selection based on the phenotype of individual plants ; open-pollination.
1. For one sex	All plants in the population allowed to produce pollen ; open-pollination.
2. For both sexes	Inferior plants detasselled ; open-pollination among the remaining plants
3. Stratified	Field divided into small plots of about 40 plants each ; selection within small plots ; open-pollination ; selection usually for one sex
4. Contiguous control	Plants of a constant genotype (single cross, inbred) used as check and planted after every 2-4 hills for comparison ; check plants detasselled ; others open-pollinate.
B. Family selection	Selection based on means of individual plant progenies or families.
1. Half-sib	Plants within each family (individual plant progeny) are half-sibs, *i.e.*, have one parent (usually the female) in common.
a. Ear-to-row	Families produced by open-pollination ; selection within superior families ; no replicated trial ; unrestricted open-pollination among all the families.
b. Modified ear-to-row	As in ear-to-row ; superior progenies identified by replicated yield trial ; pollen source—a random bulk of all the families

c. Half-sib selection	As in the modified ear-to-row, but only superior progenies planted in the crossing block allowed and to open-pollinate.
d. Modified half-sib	Half-sibs used for yield trial : S_1 families from plants producing superior half-sibs intermated through open-pollination.
e. Broad base testcross	Half-sib families produced by crossing the selected plants to a tester with a broad genetic base (parental or unrelated) used for yield trial ; S_1 progenies from plants producing superior half-sib families intermated (Syn., recurrent selection for GCA).
f. Narrow base testcross	As in the broad base testcross, but the tester has a narrow genetic base (Syn., recurrent selection for SCA).
2. Full-sib	Plants within each family are full-sibs ; produced by mating the selected plants in pairs.
a. Full-sibs intermated	Full-sibs used for yield trial ; superior full-sibs intermated.
b. S_1 progenies intermated	Full sibs used for yield trial ; S_1 progenies from the plants producing superior full-sibs intermated.
3. Inbred or Selfed	Families produced by selfing.
a. S_1	Families produced by one generation of selfing used for evaluation ; superior families intermated (Syn., simple recurrent selection)
b. S_2	Families obtained by two generations of selfing used for evaluation ; superior families intermated.
INTERPOPULATION IMPROVEMENT	Two populations improved simultaneously for combining ability with each other.
A. Half-sib reciprocal recurrent selection (HS-RRS)	See the description for reciprocal recurrent selection given in the text, Two modifications suggested by Paterniani (the second modification requires at least two ears/plant)
B. Full-sib reciprocal recurrent selection (FS-RRS)	Each selected plant in the populations A and B is selfed. Each selected plant from A is test crossed with one selected plant from B. The testcrossed progenies evaluated in field trial S_1 families of plants from A producing the superior testcross progenies intermated ; the same is done for those from B. Require at least two ears/plant in one of the two populations.

Modifications of Mass Selection

The two defects of mass selection, *viz.*, (i) lack of control on the pollen source and (2) the confusing effect of environment on the phenotypes of individual plants, may be corrected by suitable modifications of the selection scheme ; these modifications are briefly described below.

1. Inferior plants in the field are detasselled, and the remaining plants are allowed to open-pollinate. This modification exercises some control on the pollen source, but the identification of inferior plants, of necessity, is based on only those characters which are expressed before flowering.

2. Pollen from all the selected plants is collected and bulked ; this pollen is used to pollinate the selected plants. This scheme ensures full control on the pollen source, but it can be applied to only those characters which can be selected for before pollen shedding.

3. *Stratified Mass Selection.* This modification, suggested by Gardner in 1961, is also known as the *grid method of mass selection.* The field from which selection is to be done, is divided into several small plots, *e.g.*, having 40-50 plants each. Equal number of superior plants are selected from each of the plots, *i.e.*, selection is done within the plots and not among the plots. The seed from all the selected plants is composited to raise the next generation. The basis for this modification is the consideration that variation due to environment, including heterogeneity in soil fertility, will be much smaller within the small plots than that in the whole field. Thus selection within the plots is expected to be more effective than that without any stratification. Stratified mass selection has been able to increase the yielding ability of an open-pollinated variety of maize, Hays Golden, by about 3% per cycle (or generation) for 15 generations.

4. Plants of a constant genotype, *e.g.*, a single cross hybrid, are planted as checks after every one, two or four plants of the variety under selection. The yields of the plants under selection are expressed as per cent of the yield of the nearest check plant. This scheme is designed to minimise the environmental influence on the yields of the plants being selected. It employs the principle of contiguous control discussed earlier in some detail (Chapter 12).

Effectiveness of Mass Selection

The results from selection studies using mass selection have been summarised by Hallauer and Miranda (1981). Mass selection has effectively improved characters with high heritability in maize, *e.g.*, ear height, lodging resistance, ear type, adaptiveness, oil and protein contents, resistance to *Helminthosporium* leaf blight, days to flowering (tasselling as well as silking). prolificacy and stalk volume, and in other crop species. Mass selection, particularly with stratification, has also been effective in the selection for increased as well as decreased yields in maize. The results from 26 studies on mass selection for higher yields are summarised in Table 18.2. The yield increased at an average rate of 3.5% per cycle of selection ; the increases ranged from —1.0 to 19.1 per cent/cycle, the most common estimates being 1.1 to 3.4% per cycle. The gains under selection

for other quantitative characters, *e.g.*, prolificacy and ear height, appear to be relatively more than those for yield possibly due to a lower heritability for the latter. Selection for prolificacy, *i.e.*, increased number of ears per plant, as well as that for single-eared plants is highly effective in changing the yields of the selected populations as well. It is not surprising since prolificacy has a high positive correlation with grain yield in maize.

Table 18.2. Effectiveness of mass selection in changing yield and yield traits in maize.

Criteria of selection	*Number of reports*	*Selection cycles*	*Change in the character under selection/cycle (%)*		
			Mean	*Range*	*Most common estimates*
			Yield		
Increased	26	2-15	3.5	-1.0-19.1	1.1-3.4
Decreased	2	1	8.2	0.7 & 15.7	
			Prolificacy		
Increased	11	1-11	4.7	2.0-11.4	2.0-4.4
Decreased	2	1 & 11	4.5	1.5 & 7.5	
			Ear Height		
Increased	2	5 & 6	5.2 cm	2.5 & 7.9 cm	
Decreased	6	4-12	3.5 cm	2.0-7.9 cm	2.0-3.2 cm
			Ear Length		
Increased	1	10	1.6		
Decreased	1	10	3.2		
			100-Seed Weight		
Increased	1	9	2.0		
Decreased	1	9	2.0		

SELECTION WITH PROGENY TEST

Progeny Selection

The simplest form of progeny selection is the ear-to-row method which has been extensively used in maize. This method was developed by Hopkins in 1908. In its simplest form, the ear-to-row method of selection is as follows (Fig. 16.2, Scheme I).

1. A number (50-100) of plants are selected on the basis of their superior phenotype. They are allowed to be open-pollinate, and the seeds from individual plants are harvested separately.

2. A single row of 10-50 plants, *i.e.*, a progeny row, is grown from each selected plant. The progeny rows are evaluated for desirable characters and superior progenies are identified.

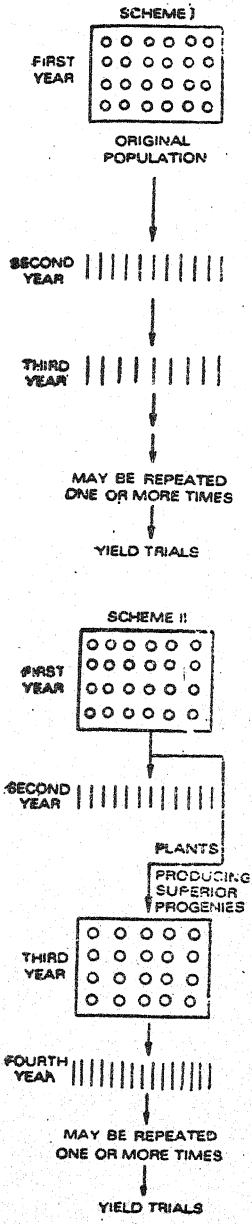


Fig. 18.2. Ear-to-row method of progeny selection as applied to maize. These schemes are termed as recurrent selection by many plant breeders who apply the term to any selection scheme which has two or more selection cycles.

3. Several phenotypically superior plants are selected from the superior progenies. There is no control on pollination ; the plants are permitted to open-pollinate.

4. Small progeny rows, as in item 2, are grown from the selected plants, and the process of selection is repeated.

It should be seen that this scheme is relatively simple and that the selection cycle is of one year only. However, it suffers from the defect that the plants in the superior progenies are pollinated by those in the weak and inferior progenies as well. This reduces the effectiveness of selection. To overcome this defect, the following modification may be used (Fig. 16.2, Scheme II).

First Year. Several plants are selected on the basis of phenotype (as in item 1 above). Open-pollinated seed is harvested separately.

Second Year. Small progeny rows are grown (as in item 2 above) and evaluated. The remaining seed from the selected plants is kept separately. Superior progenies are identified.

Third Year. The remaining seed from the plants that produced the superior progenies (identified in the second year) is bulked to produce the next generation. Plants are allowed to open-pollinate. A number of plants are selected on the basis of phenotype and the selection cycle may be repeated one or more times.

This modification of ear-to-row method was widely used in breeding of maize in U.S.A. and was responsible for the development of several varieties. In this method, plants from superior progenies only are allowed to mate among themselves. But for each selection cycle, two years are required as compared to only one in the case of ear-to-row method.

Modifications. Several modifications of ear-to-row method of selection are available and many more may be devised to suit the specific needs of breeder. Some of the modifications are briefly described below.

1. Seeds for progeny testing are obtained by selfing the selected plants and not from open-pollination. This variation is the basis for the simple recurrent selection, and is also known as S_1 *family selection*. When the seeds for progeny test are obtained after two generations of selfing, the scheme is known as S_2 *family selection*.

2. The seeds for progeny testing are obtained by crossing the selected plants to a common tester. The common tester may be an open-pollinated variety, a hybrid or an inbred. This variation has been refined as recurrent selection for general and specific combining abilities, included in the *half-sib family selection*, and reciprocal recurrent selection.

3. The progeny test consists of a replicated yield trial in place of a single row. In this method environmental effects can be separated and the actual value of each progeny can be more accurately estimated. This modification was proposed by Lonnquist in 1964, and is by far the most successful method of

progeny selection. In this case, progenies from the selected plants are planted in a replicated yield trial as well as in a crossing block (recombination or seed production plot). The progenies in the crossing block are detasselled ; they are pollinated by the pollen from the rows of a random bulk of all the selected progenies planted after every 2-3 progeny rows. Superior progenies are identified on the basis of the yield trial. Best plants from the superior progenies in the crossing block are selected, and their seeds are harvested separately. Progenies from the selected plants are handled in the same way as outlined above. In this scheme (1) the evaluation of progenies is based on a replicated trial, (2) the source of pollen is controlled, and (3) each selection cycle is completed in one year. This scheme is commonly known as *modified ear-to-row method.*

4. Seeds for progeny test are obtained by mating the selected plants in pairs so that the plants within a progeny are full-sibs, *i.e.*, have both the parents in common. This is commonly known as *full-sib family selection.*

Merits of Progeny Selection. The progeny selection schemes, except those for recurrent selection, have the following advantages.

1. In progeny selection, the selection is based on progeny test and not on the phenotypes of individual plants. The progeny test is a far more accurate reflection of the genotype than the phenotype. Thus progeny selection is far more efficient than mass selection in the identification of superior genotypes. This is a powerful tool for increasing the yielding ability of open-pollinated varieties of maize ; the yielding ability increased at the rate of 3-8 per cent per selection cycle in the different experiments.

2. Inbreeding may be avoided if care is taken to select a sufficiently large number of plant progenies and if the selected progenies are not closely related.

3. The selection scheme is still relatively simple and easy ; but some of the modifications are more complicated and tedius.

Demerits of Progeny Selection. The progeny selection schemes other than recurrent selection suffer from the following defects.

1. In most progeny selection schemes, there is no control on pollination and plants are allowed to open-pollinate. Thus the selection is based on the maternal parent only. This reduces the efficiency of selection.

2. Many of the progeny selection schemes are complicated and involve considerable work.

3. The selection cycle is usually of two years, *i.e.* the complete selection process takes two years. Thus the time requirement for selection is twice as much as that in the case of mass selection.

Applications of Mass and Progeny Selections. Mass selection has been extensively used for the improvement of cross-pollinated

crops. Even in the case of maize, where it was almost completely replaced by heterosis breeding, there is a renewed interest in population improvement. Heterosis breeding, as would be seen later, is expensive, time and labour consuming, complicated and requires strict pollination control. As a result, in a vast majority of cross-pollinated crops heterosis breeding is not likely to become economically feasible. In such cases, mass selection or one of the other population improvement schemes will remain the common breeding method. Mass selection has been used to maintain purity of the varieties of cross-pollinated crops ; in some crops, progeny selection is used for the same purpose. The population improvement schemes have been and are being used for the development of new improved open-pollinated varieties of many cross-pollinated crops.

Achievements with Mass and Progeny Selections. Mass and progeny selections have been extensively used for the improvement of cross-pollinated crops. The early varieties of bajra were developed through mass selection. Some of the examples are Babapuri, Jamnagar Giant, AF 3, S 350 and Pusa Moti; all these varieties were isolated from African introductions. Pusa Moti (developed at IARI, New Delhi) has a wide adaptability and was a popularly grown variety. Mass selection improved the yielding ability of toria (*B. campestris* var. toria) by 30 per cent and oil content by 56 per cent ; a further increase of 16 per cent in yield was obtained by using mass-pedigree method. Many early, erect to semierect types have been developed in rai (*B. juncea, e.g.*, Type 11, L16), toria (*e.g.*, Abohar), yellow sarson (*B. campestris* var. yellow sarson. *e.g.*, T 42, T 16) ; and brown sarson (*B. campestris* var. brown sarson, *e.g.*, 17 dwarf, 17 medium, DS 1 and DS 2). Selections DS 1 and DS 2 gave 251 and 286 per cent more yield than the local variety, were earlier by 5-10 days and had larger seeds. They can be planted as late as the first week of December.

Varieties have been developed through mass selection in maize (*e.g.*, T 41, T 19, Jaunpuri etc.), cotton [*e.g.*, C 402, C 520, C 1281 and K 12 from desi cotton (*G. arboreum*), and 100 F, 216 F and A 19 from American cotton (*G. hirsutum*)], castor (*R. communis, e.g.*, S 20, B 1 and B 4) and in many other crops. A large proportion of the present area under maize is planted to open-pollinated varieties which are the products of mass selection. For example, Jaunpuri maize is very popular in areas around Jaunpur and is a successful open-pollinated variety. These examples are a small sample of the vast range of achievements through mass selection.

Recurrent Selection

The idea of recurrent selection was first suggested by Hayes and Garber in 1919 and independently by East and Jones in 1920. However, cohesive breeding schemes of recurrent selection were developed during 1940s, particularly after 1945 when Hull suggested that recurrent selection may be useful in improving specific combining ability. The recurrent selection schemes were devised in relation

to heterosis breeding. The idea was to ensure the isolation of superior inbreds from the populations subjected to recurrent selection for their ultimate utilization in the production of hybrid and synthetic varieties.

The probability of isolating an outstanding inbred line depends primarily on two factors : (1) the proportion of superior genotypes present in the base population from which inbreds are isolated, and (2) the effectiveness of selection during the inbreeding process in increasing the frequency of desirable genes or gene combinations. The available evidence clearly indicates that selection is ineffective in preventing a random fixation of genes even with mild inbreeding. Thus even with strict selection, one cannot hope to isolate an outstanding inbred line from a population in any appreciable frequency. Therefore, the only course open to the breeder is to increase the frequency of favourable genes and genotypes using a breeding scheme that would keep inbreeding to a minimum.

The recurrent selection schemes are variations of progeny selection, the difference lying in the manner of obtaining the progeny for evaluation, and in making the all possible intercrosses among the selected lines in place of open-pollination. The recurrent selection schemes are of four different types, each suited for a specific purpose. These schemes are : (1) simple recurrent selection, (2) recurrent selection for general combining ability (RSGCA), (3) recurrent selection for specific combining ability (RSSCA), and (4) reciprocal recurrent selection (RRS).

Simple Recurrent Selection. In simple recurrent selection, a number of plants with desirable phenotype are selected and self-pollinated. Separate progeny rows are grown from the selected plants in the next generation. The progenies are intercrossed in all possible combinations by hand. Equal amount of seed from each cross is composited to produce the next generation. This completes the *original selection cycle*. For recurrent selection, several desirable plants are selected from the composited population obtained from the original selection cycle; they are selected on the basis of phenotype and are self-pollinated. Progeny rows are grown and all possible intercrosses are made by hand. Equal seeds from all the intercrosses are composited to produce the next generation. This constitutes the *first recurrent selection cycle*. The population may be subjected to one or more recurrent selection cycles (Fig. 18.3).

In case the character or characters under selection can be easily and accurately measured on individual plants which are selected and selfed, the above scheme is followed as such. Some other characters can be measured from seed only, *e.g.*, oil and protein contents. In such cases, the selfed seeds from the selected plants are evaluated for the characters under selection. The seeds from the plants that are superior for the concerned character are used for planting separate progeny rows. The rest of the above scheme is followed as such without modification. It may be seen that the scheme (Fig. 18.3)

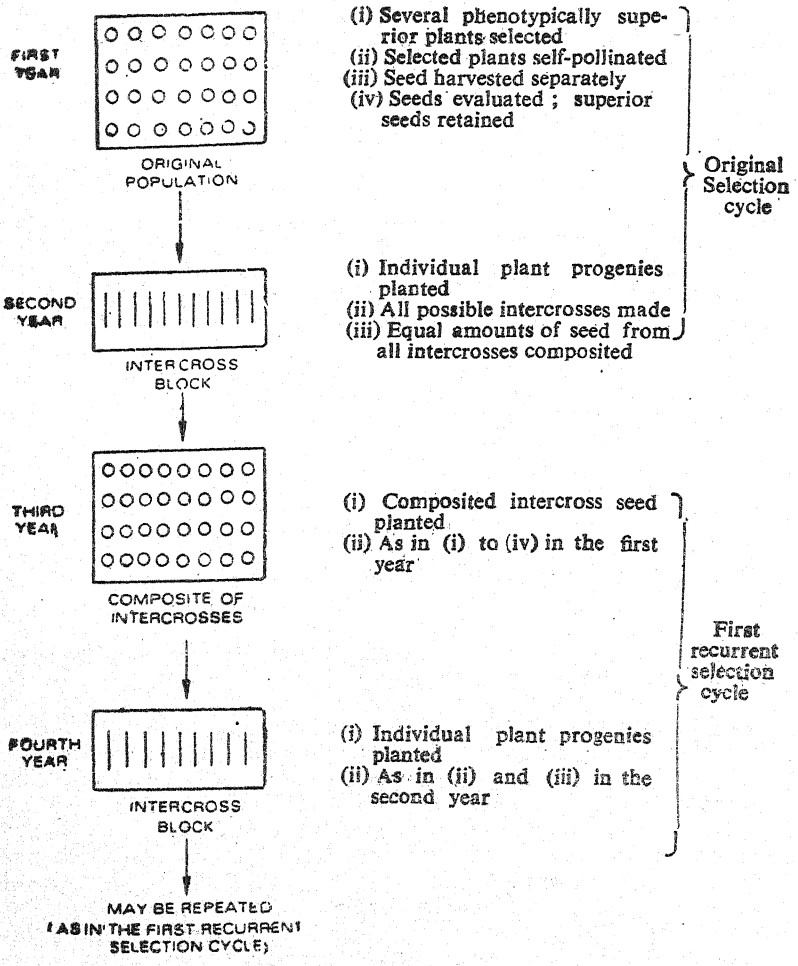


Fig. 18.3. Simple recurrent selection when the characters under selection can be easily evaluated on the basis of phenotype of the selected plants or from the selfed seed obtained from them.

is very similar to the Scheme I of progeny selection (Fig. 18.2). However, it differs from the Scheme I in two important ways : (1) the selected plants are self-pollinated as compared to open-pollination in Scheme I, and (2) the progenies are intercrossed in all possible combinations and not open-pollinated as in Scheme I.

The scheme may be modified to include progeny test for characters with low heritability, *e.g.*, yield. A portion of the self-pollinated seed from the selected plants is used to plant progeny

rows, preferably replicated, which are evaluted for the character under selection and superior progenies are identified. Next year, the remaining selfed seed from the plants producing superior progenies is planted in progeny rows for intercrossing. All possible intercrosses are made by hand. Equal seed from each intercross is mixed together to raise the next generation. This modification is very similar to the Scheme II of progeny selection (Fig. 18.2); the differences between the two are the same as those noted above.

Recurrent selection is effective in increasing the frequency of desirable genes in the population. It is the most suited for characters with high heritability. The mean of the selected population shifts in the direction of selection. Generally, there is no appreciable reduction in variability, and in some cases selected populations may show a relatively larger variation than the original population. Simple recurrent selection is considerably more efficient than selection with self-pollination. There is a relatively low inbreeding, and if care is taken it can be kept to a minimum. Inbreeding can be avoided in one of the two ways : (1) the population derived from the mixture of intercrosses may be allowed to mate at random for one generation, and this open-pollinated seed should be used to establish the population for reselection. Alternatively, (2) each intercross may be grown separately and it should be ensured that the selected plants are not related by parentage or by descent, *i.e.*, the plants are not selected from a few of the intercross only.

Recurrent Selection for General Combining Ability (RSGCA). In case of recurrent selection for general combining ability, the progeny for progeny testing are obtained by crossing the selected plants to a tester strain with a broad genetic base. A *tester* strain is the common parent mated to a number of lines, strains or plants ; such a set of crosses is used for the estimation of combining ability of the lines or plants. A tester with a broad genetic base implies a population that has a large genetic variation, *e.g.*, an open-pollinated variety, a synthetic variety or the segregating generations of a double or a multiple cross. Since the gametes from such testers would be variable, the differences between plant × tester progenies would be primarily due to the general combining ability (GCA) of the plants (the tester being common to all such progenies). It is, therefore, assumed that the plants selected on the basis of the superior performance of their plant × tester progenies would have superior GCA.

RSGCA is a direct outgrowth of the *early testing* suggested by Jenkins in 1935. *Early testing is the testing of inbreds for combining ability in the early stages of inbreeding, e.g., in the first or second selfed generation.* In 1940 Jenkins proposed a scheme for developing synthetic varieties from short-term inbreds ; this scheme is essentially RSGCA. The various steps involved in RSGCA are schematically represented in Fig. 18.4, and are briefly outlined below.

First Year. A number of phenotypically outstanding plants are selected from the source population. The source population may be an open-pollinated variety, a synthetic or an advanced generation of

a hybrid. Each selected plant is selfed as well as crossed (as male) to a number of randomly selected plants from a tester with broad genetic base. The selfed seeds are harvested separately and saved for planting in the third year. The test-cross progeny (plant × tester progeny) from each selected plant is harvested separately and used for a replicated yield trial in the second year.

Second Year. A replicated yield trial is conducted using the plant × tester progenies. The superior progenies are identified.

Third Year. Selfed seed (from the first year) from these plants that produced superior test-cross progenies (on the basis of the yield trial of the second year) is planted in separate progeny rows in a crossing block. These progenies are intercrossed in all possible combinations. Equal amounts of seed from each intercross is composited to raise the next generation. This completes the original cycle of selection.

Fourth Year. The seed obtained from bulking of all the intercrosses is planted as the source population for the first cycle of recurrent selection. Several plants are selected on the basis of their phenotype ; they are selfed as well as crossed (as males) to a number of random plants from a tester with a wide genetic base.

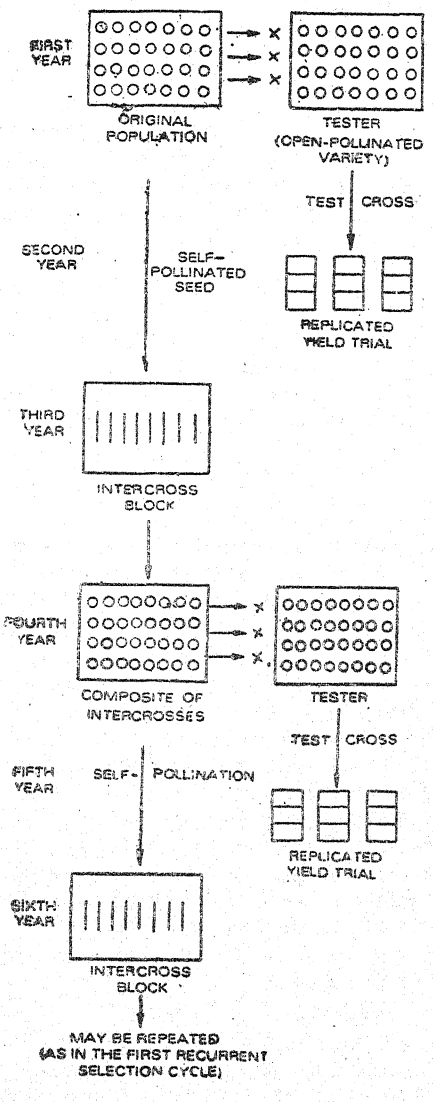
Fifth Year. Operations of the second year are repeated.

Sixth Year. Operations of the third year are repeated. This completes the first recurrent selection cycle.

Seventh Year. The second recurrent selection cycle may be initiated as in the case of the first recurrent selection cycle in the fourth year. In this manner several cycles of recurrent selection may be done.

Experimental evidence shows that RSGCA is effective in changing the GCA in the direction of selection. In addition, it is also effective in increasing the yielding ability of the population obtained at the end of the selection cycle. At the end of each recurrent selection cycle, the population is made up of equal amounts of seed from all possible intercrosses among a number of progenies selected on the basis of their general combining ability (Fig. 18.4). Thus such a population is identical with a synthetic variety and is often referred to as such. The yielding ability of a synthetic variety developed through recurrent selection may be further improved through additional cycles of selection. Generally, there is some decreasein the variability of the selected populations, most likely due to inbreeding. This loss in variability could be prevented if care is taken to avoid excessive inbreeding (see simple recurrent selection). However, in the case of characters governed by a few major genes, it may be expected that the genes would be close to fixation after a few cycles of selection leading to a drastic decrease in the variability for such characters.

RSGCA can be used for two basically different purposes. (I) It may be used to improve the yielding ability and the agronomic



(i) Several plants selected on the basis of phenotype
(ii) Selected plants self-pollinated, and
(iii) Test-crossed as males to a number of randomly selected plants from a tester with a broad genetic base

(i) Test-cross progeny from each selected plant harvested separately
(ii) Replicated yield trial is conducted
(iii) Superior progenies identified

(i) Selfed seed from plants producing superior test-cross progenies planted in a crossing block
(ii) All possible intercrosses made, and
(iii) Equal seed from all the intercrosses composited

Original selection cycle

(i) Composited seed from the intercrosses planted
(ii) As in (i) to (iii) in the first year

(i) As in the case of second year items (i) to (iii)

(i) As in the case of third year items (i) to (iii)

First recurrent selection cycle

Fig. 18.4. Recurrent selection for general combining ability. In the case of recurrent selection for specific combining ability, an inbred is used as a tester in place of an open-pollinated variety; the rest of the scheme remains the same.

characteristics of a population. In this case, the end product of selection is used as a synthetic variety. (2) Alternatively it may be used to concentrate genes for superior GCA. The end product of the selection is then used for the isolation of outstanding inbreds with superior GCA. It is expected that the frequency of such inbreds would increase after a few cycles of RSGCA.

Recurrent Selection for Specific Combining Ability (RSSCA). Recurrent selection for specific combining ability was first proposed by Hull in 1945. The objective of RSSCA is to isolate from a population such lines that will combine well with a given inbred. It is assumed that a large part of heterosis is the result of nonadditive gene action, *i.e.*, dominance and epistasis. This part of heterosis will, therefore, depend on specific gene combinations and is designated as specific combining ability (SCA). If plants are selected on the basis of the performance of their progeny derived from the testcross with an inbred, they would be selected for their combining ability with the inbred used as tester. It may be expected that these plants would have genes or gene combinations that specifically combine well with the genes present in the tester inbred. The procedure for RSSCA is identical with that for GCA, except that in this case an inbred is used as a tester in the place of an open-pollinated variety. The tester must be an outstanding inbred because it would be one of the parents of the hybrid that would be produced using the inbred lines isolated from the improved population. Therefore, great care must be exercised in the selection of the inbred to be employed at the tester in a RSSCA programme.

The selection scheme is briefly outlined below (Fig. 18.4). While referring to the Fig. 18.4, the tester should be read as an outstanding inbred.

First Year. Several plants are selected from the population and self-pollinated. The selected plants (used as males) are also crossed to an outstanding inbred used as tester (used as female).

Second Year. A replicated yield trial is planted using the testcross progeny. Outstanding progenies are identified.

Third Year. Selfed seeds from the plants that produced the outstanding progenies are planted in separate progeny-rows in a crossing block. All possible intercrosses among these progenies are made by hand. Equal amounts of seed from all the intercrosses is composited. This completes the original selection cycle.

Fourth Year. The composited intercross seed is planted and the operations of the first year are repeated.

Fifth Year. Operations of the second year are repeated.

Sixth Year. Operations of the third year are repeated. This completes the first recurrent selection cycle. The population may be subjected to one or more selection cycles, if desired, by repeating the operations of the first selection cycle.

Reciprocal Recurrent Selection (RRS). Reciprocal recurrent selection was proposed by Comstock, Robinson and Harvey in 1949. The

objective of RRS is to improve two different populations in their ability to combine well with each other. In this scheme, each of the two populations A and B serve as testers for the plants selected from the other population. For example, a random sample of plants from population A serves as the tester for the plants selected from population B ; similarly, a random sample of plants from population B serves as the tester for those selected from population A. It may be seen that this selection method allows for the selection for both GCA and SCA. It selects for GCA because the two testers (populations A and B) have broad genetic base since they are genetically heterogenous. Selection for SCA is accomplished because the two populations (or inbreds derived from them) would be crossed with each other to produce the commercial variety, and the plants in each of the two source populations are selected for their abilities to combine well with the gene combinations present in the other population, that is, for SCA with each other. A generalised scheme for RRS is outlined below (Fig. 18.5).

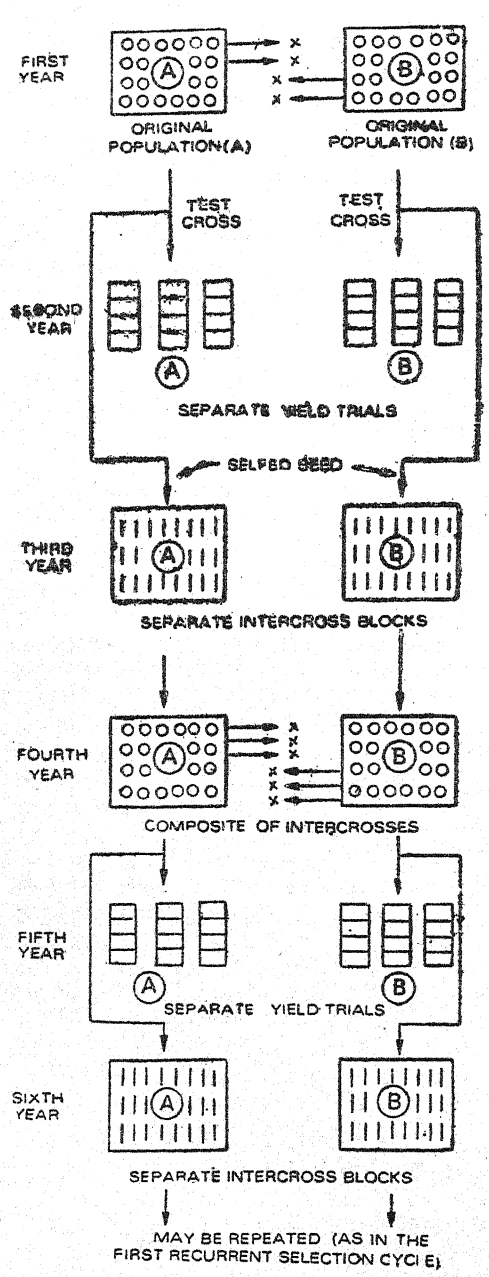
First Year. Several plants are selected from the populations A and B on the basis of their desirable phenotype. Each of the selected plants from population A is crossed as male with several randomly selected plants from the population B used as female. Similarly, each of the plants selected from the population B is crossed as male with a random sample of plants from the population A used as female. All the selected plants are selfed ; the selfed seed is harvested separately and is stored for use in the third year. All the testcross seed from an individual plant is composited and is used for progeny test in the second year (Fig. 18.5).

Second Year. Two replicated yield trials are conducted : one trial is for the testcross progenies of plants selected from the population A, and the other one is for those selected from the population B. On the basis of the progeny tests, plants producing superior progenies are identified.

Third Year. Selfed seed from the plants selected on the basis of the progeny tests (in the second year) is planted in two separate crossing blocks in individual plant-progeny rows. In one crossing block, seeds from the plants selected from the population A are planted, while in the other crossing block seeds from the plants selected from the population B are sown. Within each crossing block, all possible intercrosses among the plant-progenies are made. Equal amounts of seed from all intercrosses from the crossing block A are mixed to raise the next generation of the population A. Similarly, the next generation of population B is raised from the seed obtained by mixing equal amounts of seed from all possible intercrosses from the crossing block B. This completes the *original selection cycle.*

Fourth Year. Populations A and B are planted from the composited seed from all the intercrosses in block A and B, respectively. Operations of the first year are repeated.

Fifth Year. Operations of the second year are repeated.



(i) Several plants selected in the populations A and B
(ii) Selected plants self-pollinated
(iii) Each selected plant from A is test-crossed with several random plants from B, and vice-versa.

(i) Test-cross progeny harvested separately
(ii) Separate yield trials conducted for test-cross progenies from populations A and B
(iii) Superior progenies identified

(i) Selfed seed from plants producing superior test-cross progenies planted
(ii) All possible intercrosses are made
(iii) Seed from all intercrosses of a population mixed

Original selection cycle

(i) Composite seed from intercrosses planted separately
(ii) As in the first year items (i) to (ii)

(i) As in the second year items (i) to (iii)

(i) As in the third year items (i) to (iii)

First recurrent selection cycle

Fig. 18.5. Reciprocal recurrent selection. Two populations with broad genetic base serve as testers for each other.

Sixth Year. Operations of the third year are repeated. This completes the *first recurrent selection cycle.* The population may be subjected to further selection cycles, if desired, by repeating the operations of the first recurrent selection cycle.

The two populations developed by RRS may be used in one of the following two ways.

1. They may be intermated to produce a superior population with a broad genetic base. This is similar to a varietal cross, but in this case the populations have been subjected to selection for combining ability (both GCA and SCA) with each other. In maize (*Z. mays*), crosses between populations improved by reciprocal recurrent selection show higher yields than those between the original populations (see later).
2. Inbreds may be isolated from the populations A and B. These inbreds may then be crossed to produce a double cross or a single cross. In this case, the inbreds should be crossed in the following order.

   Single cross : $(A_1 \times B_1)$

   Double cross : $(A_1 \times A_2) \times (B_1 \times B_2)$

   where A_1 and A_2 are two inbreds isolated from the population A and B_1 and B_2 are the inbreds isolated from the population B. This would permit the maximum expression of heterosis in the double cross. There is considerable evidence that reciprocal recurrent selection increases the yielding ability of the hybrids produced by crossing the inbreds isolated from the selected populations.

Comparison between Different Recurrent Selection Schemes. On theoretical grounds, the following relationships may be expected among the three recurrent selection schemes, *viz.*, RSGCA, RSSCA and RRS.

1. When dominance is incomplete, RRS and RSGCA would be comparable in their effectiveness, and both will be superior to RSSCA.
2. When dominance is complete, the three methods would be equally effective.
3. In case of overdominance, RRS and RSSCA would be equally effective ; both would be more effective than RSGCA.
4. The above relationships are true when there is no epistasis, absence of multiple alleles and linkage disequilibrium is not present. Genetic studies with most crop species clearly demonstrate that these assumptions are unrealistic. In most situations, epistasis is important and linkage disequilibrium and multiple alleles are likely to be present. In such cases, RRS would be superior to RSGCA and RSSCA.

Thus theoretically, in almost all practical situations RRS may be expected to be superior to RSGCA and RSSCA.

Effectiveness of Progeny Selection Schemes

There is considerable literature on the effects of various progeny selection schemes on yield and yield traits in maize and other crop species. Much of the information concerning maize has been reviewed by Hallauer and Miranda (1981), and is summarised in Table 18.3. *Ear-to-row* is the earliest progeny selection scheme ; it effectively improved highly heritable traits, *e.g.*, oil and protein contents, but failed to improve yielding ability. This failure was not so much due to the deficiency of the selection scheme as due to (1) poor field plot technique during selection and evaluation, (2) contamination from foreign pollen, and (3) inbreeding depression due to small experimental populations. A welcome outcome of this failure was the developement of the hybrid varieties. The *modified ear-to-row* scheme proposed by Lonnquist in 1964 is highly effective in improving the yield of maize (average gain 4.8% per cycle).

Full-sib-family selection has been evaluated rather extensively, and is extremely effective in improving maize yields (average gain 5.3% per cycle). *Inbred* (S_1 *and* S_2) *family selections* are also effective in improving maize yields, but the rate of change per cycle appears to be somewhat less than that for modified ear-to-row and full-sib family selections (average gain 4.6 and 2.0 per cent/cycle for S_1 and S_2, respectively). *Recurrent Selections* (*RS*) *for GCA* and *SCA* effectively improved the yields of the poulations themselves (average gain 10.2 and 5.2% per cycle, respectively). These schemes also improved the GCA (measured as the yields of testcrosses) of the populations, the rate of change being slightly higher for RSGCA than that for RSSCA. *Reciprocal recurrent selection* (*RRS*) improved the yielding ability of the populations themselves as well as that of the population crosses. Some investigators, however, have reported improvement in the yields of the population crosses only, while those of the populations per se were not affected. Use of full-sibs in RRS appears to be relatively more effective than the use of half-sibs in improving the yielding ability of the two populations, but in improving the yields of the population crosses the latter appears to be more effective.

The results from different selection schemes (Table 18.3) are not strictly comparable with each other for the following reasons. *Firstly*, the different schemes were applied to different populations, and the response to selection may vary greatly from one population to another. *Secondly*, the selections and evaluations were made during different years and at different locations. Thus the E and G×E components of variation will be different for the different studies. *Lastly*, the intensities of selection were different in many of these studies, and the effectiveness of selection is greatly affected by the selection intensity. For these reasons, the results from different selection studies should be compared with considerable caution.

Comparative Effectiveness of Different Selection Schemes

On the basis of theoretical expectations, the following generali-

sations can be made. (1) The gain under selection would be proportionate to the magnitude of σ^2A in the population subjected to selection, *i.e.*, the higher the σ^2A, the greater the gain under selection. (2) As a consequence, the gains from inbred family selections would be greater than those from full-sib selection, which would be greater than those from half-sib selection schemes.

Meaningful comparisons among some selection schemes are available from 13 studies reviewed by Hallauer and Miranda (1981), and are summarised in Table 18.4. In these studies, two or more selection schemes were applied to the same population under comparable experimental and environmental conditions. *Recurrent selection for GCA* may be based on an unrelated tester or the parental population itself may serve as the tester. The use of the parental population as tester seems to be superior to the unrelated tester in improving the performance of the population per se, but it appears to be inferior to the latter in increasing the yielding ability of the testcrosses. A limited comparison is available between *RSGCA* and *RSSCA* ; the results from the four studies are contradictory. In two studies, RSSCA was superior to RSGCA in improving the yields of the populations themselves as well as that of the testcrosses. But in the remaining two studies, RSSCA was superior to RSGCA in improving the yields of the testcrosses, but it was inferior to the latter in increasing the yields of the populations per se. Thus RSSCA appears to be superior to RSGCA for improving the yields of the testcrosses (with several testers) of the selected populations, *i.e.*, in improving the GCA of the populations. This seems a little surprising because a narrow base etester is expected to select primarily for SCA.

In two studies, *S_2 family selection* was compared with *RSGCA* and *RSSCA* ; it was inferior to RSGCA in improving the yields of the populations per se, and to both RSGCA and RSSCA in increasing the yields of the testcrosses of the selected populations. This is surprising since theoretically S_2 family selection is expected to be superior to the other family selection schemes. Considerable information is available on the comparison between *RSGCA* and *S_1 family selection* ; RSGCA was marginally inferior to the latter in increasing the yields of the populations per se, but it was somewhat superior in improving the yields of the testcrosses of the improved populations. One comparison is available between *full-sib and S_1 family selections* ; full-sib selection was markedly superior to the latter in increasing the yields of the population per se.

The effectiveness of *full-sib (FS) and reciprocal recurrent selections (RRS)* was compared by Moll and coworkers in the populations Jarvis an Indian Chief. Four independent comparisons were made after 3, 3, 6 and 8 cycles of selection. In general, FS was superior to RRS in improving the yields of the two populations themselves, while RRS was superior to FS in increasing the yields of the population crosses. After 8 cycles of selection, the average gains per

Table 18.3. Effectiveness of the various progeny selection schemes in improving the yield of maize populations.

Selection scheme	*Number of reports*	*Selection cycles*		*Increase in yield/cycle (%) of the population per se*		*Increase in yield/cycle (%) of the testcrosses*	
		Mean	*Range*	*Mean*	*Range*	*Mean*	*Range*
Ear-to-row	4	8.5	7-10	0.5	−0.2-0.9		
Modified ear-to-row	6	5.8	3-12	4.8	1.8-13.6		
Broad base testcross* (RS for GCA)	19	2.1	1-4	10.2	−0.5-22.0	5.8	1.5-14.7
Narrow base testcross (RS for SCA)	17	2.8	2-5	5.2	−2.0-10.3	4.9	1.0-14.7
Full-sib family selection	12	4.2	1-10	5.3	2.5-10.6		
S_1 family selection (Simple recurrent selection)	6	3.3		4.6	1.1-6.9		
S_2 family selection	2	5.0		2.0	1.9-2.2		
Half-sib reciprocal recurrent selection	13	3.8	2-8				
Population A				3.9	−0.1-10.0	5.3	0.8-17.0**
Population B				3.6	−1.1-15.0		
Full-sib reciprocal recurrent selection	1	3					
Population A				6.0		3.2**	
Population B				5.7			

* Comparisons were made with either the original variety or a commercial hybrid, *e.g.*, US 13.

** Testcross is the cross between the two populations A and B.

Table 18.4. Effect of different recurrent selection schemes on yields of maize populations and their testcrosses. In each of these studies, two or more selection schemes were simultaneously applied to a single population.

Selection Scheme	*Number of reports*	*Selection cycles*		*Gain in yield/cycle (%) of the population per se*		*Gain in yield/cycle(%) of testcrosses*	
		Mean	*Range*	*Mean*	*Range*	*Mean*	*Range*
A. Recurrent selection (RS) for GCA							
1. Parental population as tester	8	2.1	1-4	7.7	−1.3-15.0	5.8	0.8-16.7
2. Unrelated tester	7	2.1	1-4	6.8	−1.3-25.4	7.1	0.8-18.0
B. RS for GCA	2	3.5	3-4	1·4*		5.4	3.4-7.4
RS for SCA	2	5.0	4-6	1.8*		2.2	1.7-2.6
C. RS for GCA	2	2.5	1-4	8.1*		2.4*	
RS for SCA	2	2.5	1-4	−3.2*		4.4*	
S_2 family selection	2	2.5	1-4	−2.6*		2.0*	
D. RS for GCA	9**	1.8	1-4	8.3	−1.3-25.4	7.0	0.8-18.0
S_1 family selection	8	1.9	1-4	9 9	−1.0-38.7	6.1	0.6-15.7
E. Full-sib selection	1	1		15.0			
S_1 family selection	1	1		11.0			

* Evaluations available from one study only

** In one study, RS for GCA was based on an unrelated tester as well as on the parental population used as tester.

selection cycle (expressed as per cent of the mean of the original unselected populations and their cross) were : Jarvis 3.6 and 2.5, Indian Chief 3.2 and 1.4 and Jarvis × Indian Chief 2.2 and 3.2 for the FS and RRS schemes, respectively.

In Tables 18.3 and 18.4, the gains under selection have been expressed on the per cycle basis. However, a more meaningful estimate of the gains would be on the per year basis for obvious reasons. This is particularly important when comparisons are being made among selection schemes that require different number of years per selection cycle. For example, modified ear-to-row scheme requires only one year per selection cycle, while most progeny selection schemes require 3 growing seasons/years per cycle. Therefore, the gains per year for the former shall remain unchanged, while that for most other progeny selection schemes will be only one-third of those listed in Table 17.3. Clearly the modified ear-to-row method appears to be the most efficient selection scheme for improving the yielding ability of maize populations ; the same conclusion is likely to be valid for other cross-pollinated crops as well.

General Conclusions from Selection Experiments in Maize

Selection experiments in maize have generated valuable information on the effects of selection as well as on the genetic architecture of maize populations. Some generalised conclusions based on these experiments are summarised below.

1. In general, response to selection for yield varies from one population to another, and from one selection cycle to the other. In many cases, the first or the first few selection cycles are quite effective, while the gains through subsequent selection cycles are relatively smaller. However, in some cases, the reverse may be the case.
2. All the selection schemes cause a marked reduction in the total genetic variance. This reduction generally occurs during the first few selection cycles, after which there is little change. This may explain the decreased effectiveness of the later selection cycles.
3. The major portion of genetic variance is additive in nature, and it readily responds to selection. The additive genetic variance generally shows some decline under selection, but in some cases it may show an increase.
4. A considerable additive genetic variance remains even after several cycles of selection so that : (1) there is continued response to selection, and (2) reverse selection is also effective. This is most clearly demonstrated by the studies on selection for high and low protein as well as oil contents in maize.
5. All the selection schemes mainly select for GCA, *i.e.*, additive gene effects. Even RSSCA and RRS select primarily for GCA ; there is tittle evidence that either of these schemes select for

overdominance gene effects. This conclusion is contrary to the theoretical expectations as discussed earlier.

6. RSGCA, RSSCA and RRS increase the testcross performance of the selected populations as compared to those of the unselected controls. However, the increase in heterosis (testcross performance) is mainly due to the increased yielding abilities of the populations per se. Thus the change in heterosis of the selected populations is primarily due to the selection for additive gene effects.

7. The dominance relationship in the maize populations seems to be partial to complete dominance. There is little experimental evidence to support the existence of overdominance.

8. There is some evidence that yield may reach a plateau under selection. Mass selection (stratified) in unirradiated as well as irradiated Hayes Golden open-pollinated variety of maize, and modified ear-to-row selection in unirradiated Hayes Golden improved the yield by about 40% over the unselected control after 12 and 6 cycle of selection, respectively. Four additional cycles of mass and modified ear-to-row selections failed to improve the yield any further, and surprisingly there was a marked decline during the next two selection cycles. The decrease in the yield under selection is difficult to explain, but the plateau is reminscent of that for chaetae number in *Drosophila* (Chapter 9). It would be interesting to see if reselection after a period of suspension of selection would be able to break this plateau ; if this fails, means will have to be devised to achieve this goal for a continued response to selection.

9. In general, selection for one character, *e.g.*, yield leads to changes in several other characters for which no selection is done. Such a correlated response to selection is a common feature of the selection experiments, and is most likely due to linkage among the genes governing these traits.

10. Some selection schemes, *viz.*, full sib (selfs used for intermating) and full-sib reciprocal recurrent selection, inevitably select for prolificacy. Consequently, these schemes would select for yield both directly as well indirectly. The indirect selection for yield is due to the selection for prolificacy which shows a high positive correlation with yield. Therefore, while comparing the effects of these schemes on yield with those of other schemes, this fact should be kept in mind.

SUMMARY

Population improvement aims at improving the characteristics of a population through selection. The selection may be based on phenotype without progeny testing, *e.g.*, mass selection or on phenotype as well as progeny testing, *e.g.*, progeny selection and recurrent selection. In mass selection, open-pollinated seed is collected from a number of plants with superior phenotype ; seed from these plants is composited to produce the next generation.

The selection cycle may be repeated. Mass selection is simple, takes less time and is highly effective in improving characters with high heritability. Modifications of mass selection that take into account the variation due to environment are effective in improving characters with low heritability as well. However, it is based on the female parent only.

In progeny selection, plants are selected on the basis of their phenotype and subjected to progeny testing. The progeny for progeny test may be obtained by open-pollination, self-pollination, crossing with an open-pollinated variety, a hybrid or an inbred. Superior progenies are identified ; phenotypically superior plants from these progenies are selected and subjected to progeny tests. The selection cycle may be repeated several times. There are several modified schemes of progeny selection : recurrent selection schemes are improvements on these schemes. Progeny selection is relatively simple, and is based on progeny test, but there is no control on the pollen parent, and often these schemes take a longer time than mass selection.

Recurrent selection schemes base the selection on progeny tests and exercise rigid control on pollination. The seeds for progeny tests are obtained by selfing (simple recurrent selection), or by crossing to a tester with a broad genetic base (recurrent selection for GCA) or to an inbred (recurrent selection for SCA) ; in reciprocal recurrent selection two source populations are used as testers for each other. The selected plants are selfed as well as crossed to the appropriate tester. The test cross seed is used for progeny testing. Plants producing progenies are identified by progeny tests ; selfed seed from these plants is planted in a crossing block. All possible intercrosses are made among the progenies. Equal amount of seed from all the intercrosses is mixed to produce the population for the first recurrent selection cycle. The first recurrent selection cycle consists of a repetition of the operations outlined above.

Simple recurrent selection is effective in improving characters with high heritability. Recurrent selection for GCA is highly effective in improving the GCA as well as the yielding ability of the selected populations, while that for SCA improves SCA and yielding ability. Reciprocal recurrent selection would improve GCA, SCA and yielding ability of the two source population in relation to each other. Reciprocal recurrent selection is expected to be either equal or superior to the other recurrent selection schemes under different genetic situations ranging from complete dominance to overdominance. But in most practical situations, reciprocal recurrent selection would be superior to recurrent selections for GCA and SCA.

QUESTIONS

1. Write short notes on the following : (i) mass selection, (ii) progeny test, (iii) line breeding, (iv) ear-to-row method of selection, (v) recurrent selection, (vi) population improvement, and (vii) objectives of selection in cross-pollinated crops.
2. Briefly describe the contributions of the following scientists, (i) Hopkins, (ii) Hayes and Garber, (iii) East and Jones, (iv) Jenkins, (v) Hull, and (vi) Robinson, Comstock and Harvey.
3. What is recurrent selection ? List the various types of recurrent selection. Describe one of the schemes for recurrent selection and compare the effectiveness of the various schemes.
4. Compare the efficiency of the different schemes for population improvement. Which scheme do you prefer and why ?
5. In the case of following, what breeding method(s) would be appropriate and why ? (i) characters governed by dominance gene action, (ii) characters governed by additive gene action, (iii) characters governed by epistatic interactions, (iv) characters with high heritability, (v) characters with low heritability.
6. Define the following : mass selection, progeny test, recurrent selection for SCA, tester, reciprocal recurrent selection.

7. Describe briefly the merits and demerits of the following : (i) mass selection, (ii) progeny selection, (iii) simple recurrent selection, (iv) recurrent selection for SCA, and (v) recurrent selection for GCA.

Suggested Further Reading

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CHAPTER 19

Hybrid and Synthetic Varieties

The two important features of cross-pollinated species are inbreeding depression and heterosis. Population improvement schemes generally aim at keeping inbreeding at a low level to avoid its ill effects, and an effort to exploit heterosis is rarely made. But heterosis is the basis for the breeding of hybrid and synthetic varieties. In fact, hybrid varieties are the best means of utilizing heterosis. Synthetic varieties utilize only a part of heterosis, but under certain situations they offer the only practical means for it. Hybrid and synthetic varieties have been highly successful in many cross-pollinated species, *e.g.*, maize, jowar and bajra, and in even some self-pollinated species, *e.g.*, cotton, rice and tomato. In India, almost all the recommended varieties of maize today are either hybrid or composite varieties. As in the other chapters on cross-pollinated crop species, more is known about hybrid and synthetic varieties in maize than in all the other crops combined. Our discussion would naturally be primarily based on the knowledge gained from maize, but it is equally applicable to other crop species.

DEFINITIONS

Hybrid varieties are the first generations (F_1) from crosses between two purelines, inbreds, open-pollinated varieties, clones or other populations that are genetically dissimilar. Most of the commercial hybrid varieties are F_1's from two or more purelines (tomato, *L. esculentum*), or inbreds (maize, *Z. mays*; jowar, *S. bicolor*; and bajra, *P. americanum*). An *inbred* is a nearly homozygous line obtained by continuous inbreeding of a cross-pollinated species with selection accompanying inbreeding, and is maintained by close inbreeding, preferably by self-pollination. When two inbreds, say A and B, are crossed to produce the hybrid (A×B), it is known as a *single cross*. When two single crosses, say (A×B) and (C×D), are crossed, the resulting hybrid population, (A×B) × (C×D), is known as a *double cross*. Thus a double cross involves four inbreds, which

are first mated to produce two single crosses ; the single crosses are then hybridized to yield the double cross. A *threeway cross* is a cross between a single cross (A × B) and an inbred (C) to yield the hybrid population (A × B) × C. When an inbred is crossed with an open-pollinated variety, it is known as an *inbred-variety cross* or a *topcross*. A topcross, however, also denotes crosses of selected plants, lines or clones with an open-pollinated variety. The purpose of the top-cross is to estimate GCA of the plants or lines crossed with the open-pollinated variety. When the cross is made to assess the combining ability, it is known as *testcross* ; a testcross may be made with an inbred (for SCA), hybrid, synthetic or open-pollinated variety (for GCA). The common parent used in the test-cross is known as *tester*, and the progeny derived from these crosses are known as *testcross progeny*. Another term that deserves mention is polycross. *Polycross* denotes the progeny of a line produced through random pollination by a number of selected lines. We shall turn back to polycross in connection with synthetic varieties. When two open-pollinated varieties are mated, it is known as *varietal* or *population cross*.

HYBRID VARIETIES

History

Hybrid varieties were first commercially exploited in maize. The use of hybrid varieties was, ironically, not prompted by the superiority of hybrids, but by the realization that mass and progeny selections were not able to improve the yielding ability of open-pollinated varieties to any substantial extent. As early as 1878, Beal had shown that certain varietal crosses showed substantial heterosis; he suggested that such varietal hybrids may be used as varieties. Varietal hybrids were indeed grown commercially on a small scale. In 1909, Shull suggested that inbreds should be developed from open-pollinated varieties by continued self-fertilization. The inbreds that combined to produce superior hybrids should then be crossed to produce single cross hybrid varieties. Shull's scheme could not be exploited commercially because of the following reasons :

1. Outstanding inbreds that would produce hybrids with yielding abilities substantially higher than those of open-pollinated varieties were not available.

2. Since the female parent was an inbred, the amount of hybrid seed produced per acre was low (30-40 per cent of the open-pollinated varieties). *Consequently, the hybrid seed was expensive.*

3. The male parent was also an inbred ; hence pollen production was poor. Consequently, more area had to be planted under the male parent. *This made the hybrid seed more expensive.*

4. The hybrid seed was often poorly developed as it was produced by an inbred and had a relatively poor germination making a higher seed rate necessary.

The last three of the above difficulties were overcome by the double cross scheme proposed by Jones in 1918. Since in a double cross the female as well as the male parents are single crosses, seed and pollen production is abundant, seed quality and germination are high, and, as a result, the cost of the hybrid seed is low. The idea of double cross was adopted soon after it was proposed ; the first commercial variety was released in 1921. Popularisation of commercial hybrid varieties in U.S.A. was slow, but by 1944 about 80 per cent of the area under maize was under hybrid varieties. It is believed that in U.S.A. hybrid varieties represented, on an average, an yield increase of about 20 per cent over the open-pollinated varieties. In India, this increase has been estimated to be still higher, *viz.*, 30-50 per cent.

The utilization of cytoplasmic-genetic male sterility is one of the significant landmarks in the development of hybrid varieties. The Texas cytoplasmic male sterility was identified in 1938, and it was commercially exploited for hybrid seed production during late 1960s. Subsequently, population improvement of the source populations and the improvement of inbreds markedly increased the yielding ability of the inbreds. As a result, by 1960 single crosses had become widely accepted in U.S.A. at the expense of double crosses. Today in U.S.A., single cross maize hybrids have virtually replaced double cross varieties.

In India, intensive research for the development of hybrid maize began in 1952 when the Coordinated Maize Improvement Project began in collaboration with the Rockefeller Foundation. A large number of inbeeds and other germplasm were introduced from U.S.A. ; the inbreds were used for the production of hybrid maize (*Z. mays*). Attempts to isolate superior inbreds from the indigenous germplasm have not been much successful. Four hybrids were released in 1961 ; these were Ganga 1, Ganga 101, Ranjit and Deccan. Subsequently, in 1961, work on hybrid varieties in jowar (*S. bicolor*) and bajra (*P. americanum*) was taken up ; these were based on the cytoplasmic-genetic male sterility of Combine Kafir 60 and Tift 23A, respectively. The first hybrid variety of jowar, CSH 1, was released in 1964, while that of bajra, HB 1 (HB=hybrid bajra), was released a little later in the same year. However, some bajra hybrids developed without the use of male sterility were released much earlier than this, *e.g.*, hybrid X_1 and hybrid X_3 in 1949 (Bombay State, 25 per cent higher yield over the best varieties), and hybrid 28-15-1×AF-3-9-1 in 1955 (Maharashtra, 10 per cent more yield).

Hybrid maize could not become very popular in India for several reasons. The foremost reasons were the intensive management and input requirements, and the need for changing the seed every year. Subsequently, the development of *synthetic* or *composite* varieties was undertaken ; in 1967 six composite varieties, *e.g.*, Vijay, Kisan, Amber etc., were released. Today the emphasis of the

Coordinated Maize Improvement Project is on the development of composite varieties and on population improvement. But in jowar and bajra, hybrid varieties remain the breeding objective.

Operations in the Production of Hybrid Varieties

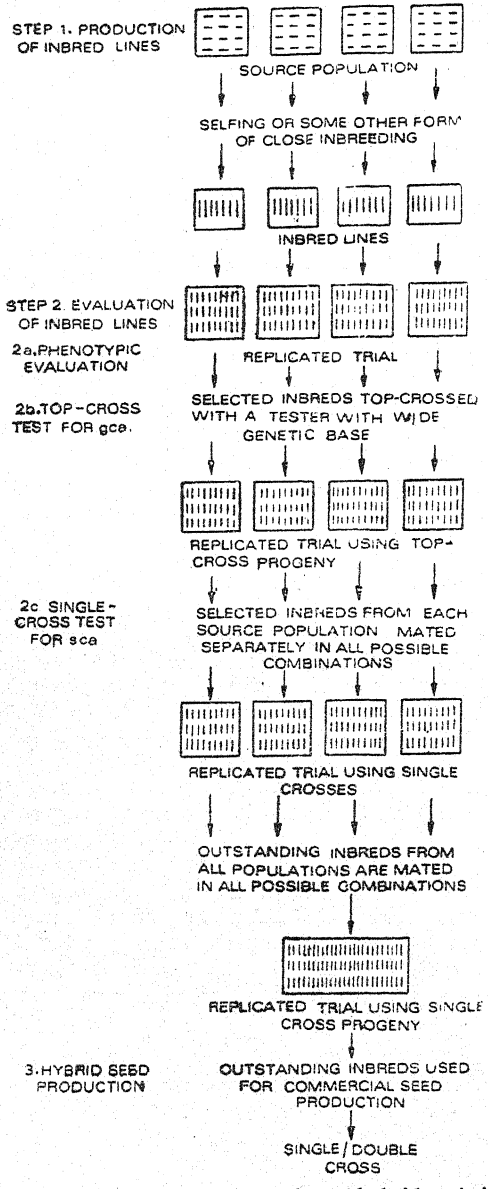
In the production of commercial hybrid varieties of sexually propagated species, inbreds are highly desirable in comparison to open-pollinated varieties or other populations with a broad genetic base. This is because (1) inbreds can be maintained indefinitely without a change in their genotype, while the genetic make-up of open-pollinated varieties is likely to be modified by the evolutionary forces (Chapter 8) ; (2) the hybrids derived from inbreds are homogeneous, or nearly so, year after year, while those produced from open-pollinated varieties are likely to be variable, and hence their performance cannot be accurately predicted ; (3) the uniformity of the inbred-derived hybrids is also desirable from the viewpoint of uniform quality of the produce. For these reasons, hybrid varieties in case of maize and other crops are produced almost exclusively from inbred lines. The operations involved in the production of hybrid varieties in such a case are : (1) development of inbred lines, (2) evaluation of inbreds, and (3) production of hybrid seed (Fig. 19.1).

Development of Inbreds

Inbred lines are developed from a genetically variable population by continued inbreeding. The population from which inbreds are isolated is the *source population*. The source population is generally an open-pollinated variety, but it may as well be a synthetic, a single cross or a double cross. Inbreds isolated from an open-pollinated variety, which may or may not have been subjected to population improvement, are known as *first cycle inbreds*. On the other hand, inbreds isolated from hybrid varieties are termed as *second, third* or *fourth cycle inbreds* depending upon the number of improvement cycles (0, 1 and 2, respectively) the inbreds making-up the hybrid varieties have been subjected to. The inbreds isolated from synthetic varieties may or may not be second cycle inbreds. If a synthetic variety is derived from inbreds, the inbreds isolated from it would be second or higher cycle inbreds, otherwise not. The development of second cycle inbreds is considered later under 'Improvement of Inbreds'. The production of *first cycle inbreds* is briefly outlined here.

Isolation of Inbreds by Inbreeding. Inbreds are developed by a suitable system of close inbreeding. But self-pollination is desirable, wherever possible, as it leads to homozygosity very rapidly. The procedure for the isolation of inbreds through self-pollination is described below.

First Year. A number of plants with desirable phenotypes are selected from a source population and are self-pollinated. The selected plants should be vigorous and free from diseases. They may be selected on the basis of their GCA estimates obtained by testing the



Open-pollinated plants from diverse source populations are subjected to continuous inbreeding with selection. After 6-7 generations of self-pollination, nearly homozygous inbred lines are obtained.

Selection among inbreds based on phenotypic evaluation from a replicated trial. Very poor inbreds are discarded.

Inbreds tested for GCA on the basis of topcross test. About 50 per cent of inbreds rejected on the basis of the performance of topcross progeny.

The remaining inbreds are subjected to single cross test for SCA. Inbreds from different source populations are evaluated separately for economy.

Few outstanding inbreds from each of the populations are pooled and crossed in all possible combinations. Outstanding single crosses are used for estimating the performance of various double crosses.

The final step consists of the production of the outstanding single or double cross at a commercial scale.

Fig. 19.1. Steps in the production of hybrid varieties in cross-pollinated crops, based on the present practice in maize.

performance of their testcross progeny (the tester should have a wide genetic base). Experimental evidence clearly reveals that open-pollinated (S_0) plants differ in their GCA and that GCA can be successfully selected for.

Second Year. About 30-40 plants are space planted from the selfed seed from each of the selected plants. Best plants are selected from the best progeny rows and are self-pollinated.

Third to Sixth Years. The process of the second year is repeated. But as the number of generations of self-pollination increases, individual plant progenies would become more and more homogeneous. Consequently, in the later phases of inbreeding selection is primarily among the progenies rather than within the progenies.

Most of the material would be discarded due to deficiencies and weaknesses, but a few outstanding lines would be maintained. These lines would be the inbreds that might be useful in a hybrid programme.

Seventh Year. At this stage, individual plant progenies would be almost homogeneous as they would be expected to be nearly homozygous. Selfing may be discontinued and the inbreds are generally maintained by sib-pollination.

Development of Inbreds from Haploid Plants. In maize, haploid plants occur spontaneously at a low frequency ; the frequency varies from 10^{-3} in most of the strains to 10^{-2} in certain genotypes. These haploids originate due to the parthenogenetic development of female gamete. Therefore, the identification of such haploids becomes very easy if a pollinator carrying a dominant seedling marker is used ; all the plants in the progeny lacking this marker are likely to be haploids. Haploids are sterile, but about 10 per cent of the haploids set some seed due to spontaneous chromosome doubling in small sectors. The diploid progeny from haploids are homozygous and serve as instant inbreds. This method presents a short-cut to the lengthy inbreeding programmes for isolating inbreds. Haploid plants may also be obtained by parthenogenetic development of the male gamete, but the frequency of such haploids is very low.

A substantial number of inbreds developed by the haploid technique has been evaluated for combining ability. They are comparable to a random sample of the conventional inbreds developed by selfing.

Selection During Inbreeding. Generally, a strict selection is practised during inbreeding. Close inbreeding leads to a rapid and random fixation of genes and does not allow the accumulation of desirable genes through recombination. It is generally accepted that selection accompanied with close inbreeding is more or less ineffective in increasing the frequency of desirable genes. But selection is highly effective for characters with high heritability, and in eliminating weak and undesirable lines. Thus selection during inbreeding is ineffective in improving the combining ability of inbreds, but is effective

in improving the performance of inbreds themselves, which is an important factor in hybrid seed production.

Early Testing. As pointed out earlier, *GCA of inbreds can be effectively tested at an early stage during the inbreding programme ; this is known as early testing.* Early testing was proposed by Jenkins in 1935. In early testing, open-pollinated (S_0) plants or first or second generation selfs (S_1 or S_2) may be subjected to the test for GCA. The procedure for early testing is simple. *First*, several S_0, S_1 or S_2 plants are selected on the basis of phenotype and are self-pollinated. These plants are simultaneously testcrossed with a tester with a wide genetic base. The *second step* consists of planting individual plant progenies from the selfed seed and a replicated yield trial is planted for the testcross progenies. The performance of testcross progenies is the basis for the identification of superior S_0, S_1 or S_2 plants ; the selfed progenies from such plants are maintained by self-pollination, while the progenies from inferior plants are rejected. The test for GCA may be repeated and used as the basis for selection of superior or desirable plants in the early inbreeding generations. *There is some evidence that selection during inbreeding based on the performance of testcross progeny is highly effective in improving the GCA of inbreds.* Early testing has been widely used in hybrid programmes, particularly in those species where inbreds are rarely, if ever, developed, *e.g.*, in forage crops and clonal crops.

Evaluation of Inbreds

If all the inbreds developed from an open-pollinated variety were mated at random, the average yield of all the single crosses would be the same as that of the open-pollinated variety. This is because it amounts to random mating after several generations of self-pollination in a Mendelian population. We have already seen that visual selection during the isolation of inbreds is not likely to affect, to any appreciable extent, the performance of hybrids derived from them. This is self-evident from the fact that of the more than 100,000 inbreds tested till 1950 in U.S.A., only 60 were fit for commercial exploitation. Thus the most important operation in a hybrid programme is the identification of inbreds that would produce an outstanding hybrid suitable for commercial use. And undoubtedly it is the most expensive operation in the development of hybrid varieties. If n inbreds are to be tested in all possible single cross combinations, there would be $n\,(n-1)/2$ single crosses that must be evaluated in replicated yield trials. With $n=20$, the number of single crosses would be 190. But the number of double crosses from n inbreds is prohibitively large, that is, $3 \times n!\,[(4!)\,(n-4!)]$, which for 20 inbreds would be 14,535. Therefore, double cross combinations of inbreds are not evaluated, except for release as a variety. The modern practice of inbred evaluation may be divided into the following four steps : (1) phenotypic evaluation, (2) top-cross test for GCA, (3) single cross test for SCA, and (4) prediction of double cross performance from the data on the performance of single

crosses. *These steps, briefly outlined below, are followed in the given order.*

Phenotypic Evaluation. It is based on the phenotypic performance of inbreds themselves. It is highly effective for characters with high heritability, *i.e.*, high GCA. To some extent, it is effective in improving the yielding ability of hybrids as the yield of inbreds shows a small (usually 0.2) but positive correlation with the performance of their hybrids. Thus inbreds with very poor performance can be safely rejected. The performance of inbreds is tested in a replicated yield trial, and the inbreds showing poor performance are discarded.

Topcross Test. It is generally accepted that the performance of topcross progeny of an inbred is a reliable measure of the average performance of all the single crosses involving an inbred, and that topcross performance provides a reliable estimate of GCA. The inbreds remaining after the phenotypic evaluation are crossed to a tester with a wide genetic base, *e.g.*, an open-pollinated variety, a synthetic, or a double cross. A simple way of producing topcross seed in maize is to plant alternate rows of the tester and the inbreds to be tested. The inbreds are detasselled ; the seed from the inbreds is harvested and it represents the topcross seed. The performance of the topcross progeny is evaluated in replicated yield trials, preferably over locations and years. *Based on the topcross test, about 50 per cent of the inbreds are eliminated.* This reduces the number of inbreds to a manageable size for the next step.

Single Cross Evaluation. It is believed that an important part of heterosis is due to SCA. Hence the outstanding single cross combinations can be identified only by testing the performance of single crosses. The final evaluation of the inbreds, therefore, consists of the evaluation of single crosses produced from them. The inbreds remaining after the topcross test are generally crossed in a diallel manner to produce all possible single crosses (reciprocals are not included). In a *diallel system of mating*, each inbred is crossed with every other inbred. This produces $n\ (n-1)$ single crosses if the reciprocal crosses are also made. and $n\ (n-1)/2$ single crosses if the reciprocals are ignored.

The performance of single crosses is evaluated in a replicated yield trial, preferably over years and locations. Outstanding single crosses are identified and may be released as hybrid varieties where production of single crosses is commercially feasible. More commonly, *e.g.*, in the case of maize the performance of single crosses is used to predict the performance of double crosses.

Prediction of Double Cross Performance. Prediction of double cross performance from the data on single crosses is a widely accepted practice. Not only this saves enormous time, labour and money, it is surprisingly accurate. *The predicted performance of any double cross is the average performance of the four nonparental single crosses involving the four parental inbreds.* Suppose, we wish to predict the

performance of a double cross involving the four inbreds. A, B, C and D. The six possible single crosses among these inbreds would be A×B, A×C, A×D, B×C, B×D and C×D. These single crosses can be combined to produce 3 double crosses, *viz.*, (A×B)×(C×D), (A×C)×(B×D) and (A×D)×(B×C). The performance of any of these double crosses can be predicted from the performance of the four single crosses not involved in producing that particular double cross. For example, the performance of the double cross (A×B)×(C×D) would be the average of the performance of the single crosses A×C, A×D, B×C and B×D, since these single crosses are not involved in producing this double cross. Similarly, the performance of the double cross (A×C)×(B×D) can be predicted from the average of the performances of the single crosses A×B, A×D, B×C and C×D. Which single crosses would be used to predict the performance of the double cross (A×D)×(B×C) ?

The *order of inbreds in a double cross* is decided by their genetic relationship. Inbreds that are genetically related, *i.e.*, derived from the same or related sources, are used to produce the single crosses. Genetically unrelated single crosses are then mated to produce the double cross. For example, if inbreds A and B are from one source, while C and D are from another, A would be crossed with B, and C would be mated with D to produce the single crosses A×B and C×D. These single crosses would then be mated to produce the double cross (A×B)×(C×D) ; this would be the best double cross from these four inbreds.

Production of Hybrid Seed

The two requirements of commercial hybrid seed production are : (1) easy emasculation of the female parent, and (2) effective pollen dispersal from the male parent to ensure a satisfactory seed set in the female parent. Both these factors are largely governed by the floral structure and the natural mode of reproduction of the crop species in question. For example, emasculation in maize, due to its peculiarly favourable floral structure, consists of a very simple operation of *detaselling*, *i.e.*, removal of the entire tassel (the male inflorescence) from the plant before pollen is shed. Thus one simple operation emasculates the whole plant. The production of hybrid maize till 1960s was essentially based on detasselling. However, male sterility and self-incompatibility offer the means for *genetic emasculation*, that is, preventing self-fertilization by manipulating the genotype of the plant, and are the basis of hybrid seed production in many crops. Pollen dispersal is often satisfactory in most cross-pollinated species since it is their natural mode of reproduction. But in self-pollinated species, satisfactory pollen dispersal is often the limiting factor in hybrid seed production. Hybrid seed may be produced in one of the following several ways : (1) cytoplasmic-genetic male sterility, (2) cytoplasmic male sterility, (3) genetic male sterility, (4) self-incompatibility, and (5) manual emasculation and/or pollination. The first four systems have been described in some detail in.

Chapter 3 where their merits and demerits have been discussed. The first and the fifth system have been described in Chapter 16 in relation to jowar and cotton, respectively.

Cytoplasmic-Genetic Male Sterility. This system is the most widely used method in hybrid seed production. It is commercially used in maize, bajra, jowar, onions (*A. cepa*) and sugarbeets (*B. vulgaris*). The system is based on a cytoplasm that produces male sterility and on a gene that restores fertility in the presence of the male sterile cytoplasm. The development of male sterile and restorer lines has been described in Chapter 3. The use of this system in hybrid seed production is outlined below.

Production of Single Cross Hybrid Varieties. For the production of a single cross, a male sterile line is used as female, and the male parent is a restorer. The seed set on the female parent (the male sterile line) is the hybrid seed, while that produced on the male parent is selfed seed. The resulting hybrid is male fertile since it has received the restorer gene from the male parent (Fig. 19.2). Generally, two rows of the male fertile inbred (the male parent) are planted after every two rows of the male sterile parent (the female parent). But when the male inbred produces sufficient pollen, 2 rows of the male inbred may be planted after every 3 or 4 rows of the female parent. The present single cross hybrid varieties of maize are produced by planting male and female inbreds in the ratio of 2 : 4.

Production of Double Cross Hybrid Varieties. Double cross hybrid varieties are produced by crossing two single crosses, one male sterile and the other male fertile. The male sterile single cross is produced by crossing a cytoplasmic male sterile line with a non-restorer male fertile line [Fig. 19.2, Single Cross (A × B) of the Double Cross Schemes I and II]. The male fertile single cross may be produced in one of the two ways. *First*, a cytoplasmic male sterile line is crossed with a restorer line (Fig. 19.2, Scheme I); the double cross in this case has both male sterile and male fertile plants in the ratio 1 : 1. In the *second method*, two restorer lines are crossed together ; one of the restorer lines serves as female and is detasselled manually (Fig. 19.2, Scheme II). All the plants in double cross would be male fertile. Scheme II is expensive, but it may have some usefulness in crop species where pollen production is limited. Even in maize, in certain areas pollen production may be poor making the Scheme II desirable.

Cytoplasmic Male Sterility. The scheme for hybrid seed production is the same as that with cytoplasmic genetic male sterility, except that the male fertile line is nonrestorer (by the definition of cytoplasmic male sterility system). The hybrid, therefore, is male sterile. It may be useful in crops where grain or seed is not the commercial product. It has not been exploited commercially to any appreciable extent.

Genetic Male Sterility. The male sterile line (*ms ms*) is allowed to be cross-pollinated with a male fertile line (*Ms Ms*) to yield a male

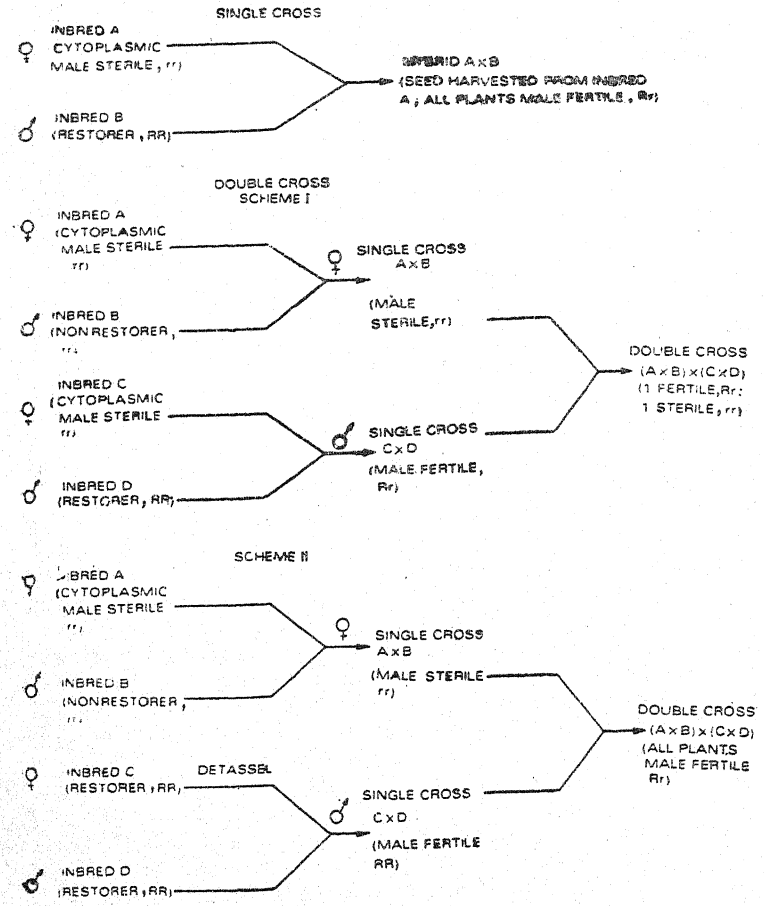


Fig. 19.2. The use of cytoplasmic-genetic male sterility in the production of hybrid seed.

fertile hybrid (*Ms ms*). The seed produced on male sterile line is the hybrid seed. The development and maintenance of male sterile lines has been discussed in some detail in Chapter 3. It has been commercially exploited in castor (U.S.A.) and pigeonpea (India, on a small scale).

Self-Incompatibility. Two self-incompatible but cross-compatible lines are planted in alternate rows; the seed produced by both the lines would be hybrid seed. Alternatively, a self-compatible line may be interplanted with a self-incompatible line. In this case, the seed from self-incompatible line will be the hybrid seed, while that

from the self-compatible line will be a mixture of hybrid and selfed seed. Therefore, the seed from self-incompatible line only is used as the hybrid variety. This system is being commercially used for hybrid seed production in some *Brassica* crops in Europe and Japan, *e.g.*, Brussel's sprouts (*B. Oleracea*) and cabbage (*B. oleracea*).

Manual Emasculation and/or Pollination. This method relies on manual emasculation and, in many cases, on manual pollination. Early hybrid maize production was based on manual emasculation, *i.e.*, detasselling. The Scheme II of double cross production (Fig. 19.2) also involves detasselling for producing the single cross (C × D). Manual emasculation and pollination has been successfully used for hybrid seed production of tomatoes in Europe, and of cotton in India. The hybrid seed produced by manual operations is very costly. Consequently, this system of hybrid seed production is limited only to those crops where the returns are very high making the production of hybrid seed economically feasible.

Improving The Characteristics of Inbred Lines

The direct isolation of inbreds from source populations and their evaluation is a time consuming and expensive process. Further, the frequency of outstanding inbreds in direct isolations is very low. As a result, various schemes have been suggested to improve the existing inbreds in respect of (1) the productivity of inbreds to make them suitable for use in hybrid seed production, (2) disease and insect resistance or some other characteristics of the inbreds so that the characteristics of the hybrid are improved, or (3) the combining ability of inbreds to increase the yielding ability of their hybrids. One or more of these objectives are fulfilled by the following methods : (1) pedigree selection, (2) backcross method, (3) convergent improvement, and (4) gamete selection.

Pedigree Selection. Pedigree selection consists of the isolation of inbreds from an outstanding single, or even a double cross. Two inbreds that complement each other for disease resistance and for other desirable attributes are crossed and in the segregating generations desirable recombinants are selected. The segregating generations are produced by selfing or close inbreeding. The method is closely similar to the pedigree method used in self-pollinated crops, as is suggested by its name. The inbreds produced in this manner are known as *second cycle* inbreds. Second cycle inbreds are considerably superior to the first cycle ones in their *per se* performance. The performance of their hybrids may also be improved, but generally the improvement is not substantial.

Backcross Method. This is essentially the same method as that applicable to the self-pollinated crops (Chapter 15), and has the same applications. An otherwise desirable inbred is used as the recurrent parent in a backcross programme. The donor parent is an inbred that has the desirable characters in a high intensity. Backcross

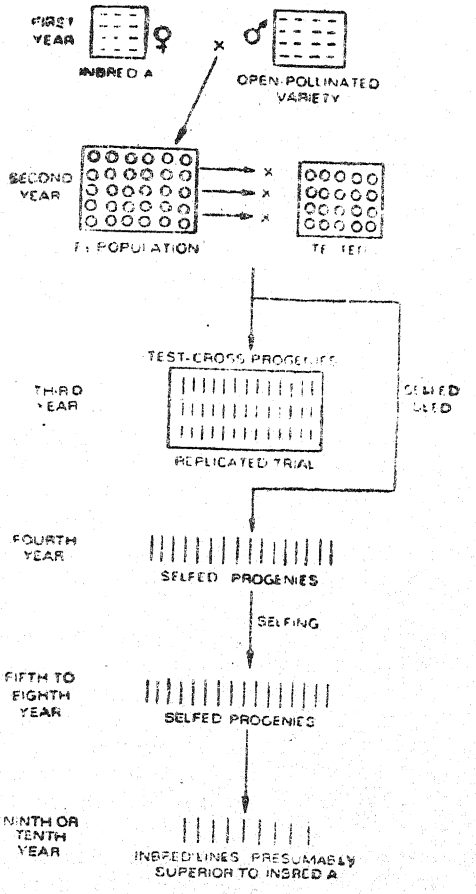
method can be used to transfer any character that has high heritability. It has been used to improve disease resistance, insect resistance, *e.g.*, resistance to stem border in maize, lodging resistance and several other characteristics of inbreds. A specialised application of the backcross method is the transfer of cytoplasmic male sterility and of restorer genes to new inbreds. An example of the improvement of inbreds through the backcross method is furnished by bajra. The male sterile line Tift 23A, introduced from U.S.A., was highly susceptible to downy mildew leading to the high susceptibility of the hybrids produced by using it as the female parent. Downy mildew resistant male sterile lines have been produced by backcrossing Tift 23A to some downy mildew resistant African and Indian lines.

Convergent Improvement. *It is a special case of backcross ; a single cross is backcrossed separately to the two parental inbreds.* Selection is made on the basis of phenotype during the backcrossing. It is hoped that the two parental inbreds, *e.g.*, A and B, would be improved by retaining some of the favourable genes contributed by the other inbred. Thus A is expected to be improved by the genes from B, and B is expected to be improved by the genes from A. There is some evidence that this method improves, to some extent, the performance of inbreds as well as that of the hybrids produced from them. But generally the improvement is not substantial, and the method has not been widely used.

Gamete Selection. Gamete selection was proposed by Stadler in 1944. The basis for this scheme is the consideration that the frequency of superior gametes (p) in a random mating population is appreciably higher than that of the superior zygotes (p^2). This is particularly so when $p<0.5$.

In *gamete selection*, a good inbred line is crossed with a random sample of gametes from an open-pollinated variety. The resulting F_1 plants are selfed as well as crossed to a suitable tester. The tester may be an inbred or a population with a wide genetic base, depending upon the objective of the programme. The testcross progeny are evaluated in a replicated trial. The differences in the performance of the testcross progenies would be entirely due to the gametes from the open-pollinated variety, since the other parent is an inbred which would be homozygous for all practical purposes. Selfed seeds from the F_1 plants that produced superior testcross progenies are planted in progeny rows (Fig. 19.3). Selfing and selection is continued to develop inbred lines which are expected to be superior to the inbred used as one of the parents.

This scheme has not been used on a large scale for the development of inbred lines. It is argued that the scheme has considerable potential and should be widely used for producing superior inbreds.



A good inbred A is pollinated with a random sample of pollen from an open-pollinated variety.

(i) F_1 is space-planted.
(ii) F_1 plants are test-crossed as well as self-pollinated.
(iii) Selfed seed is kept for use in the fourth year.

(i) Testcross progenies are planted in a replicated trial
(ii) Superior F_1 plants are identified on the basis of performance of their testcross progeny.

(i) Selfed seed from the F_1 plants that produced superior testcross progenies are planted in progeny rows.
(ii) Superior plants are selected and selfed.

(i) Selfed seed from the selected plants sown in progeny rows.
(ii) Superior plants are selected and selfed.

(i) Inbreds maintained by sibmating.

Fig. 19.3. Gamete selection in maize for the improvement of inbred lines.

Merits of Hybrid Varieties

1. Hybrid varieties exploit both GCA and SCA. component of heterosis. Thus they utilize heterosis to the greatest possible extent.

2. The produce from hybrid, particularly single cross varieties, is more uniform as compared to that from open-pollinated, synthetic or composite varieties.

3. They can be produced both in cross- and self-pollinated crop species. They are the only possible means of exploiting

heterosis in self-pollinated species, as open-pollinated, synthetic and composite varieties are not possible in them.

4. Hybrid varieties are maintained in the form of their parental inbreds, which are grown in isolation and subjected to sib-mating. This ensures that the genetic constitution of a hybrid variety does not change with time, except from some spontaneous mutations. In contrast, the genetic compositions of open-pollinated, synthetic and composite varieties are likely to change with time due to natural and unintended artificial selection.
5. In many self-pollinated crops, hybrid varieties yield 25-30% more than the pureline varieties. As a result, they are becoming popular among the farmers despite the high cost of hybrid seed (*e.g.*, Rs. 50.00 per kg. for hybrid arhar and upto Rs. 150.00 per kg. for hybrid cotton).

Demerits of Hybrid Varieties

1. Farmers have to use new hybrid seed every year. They cannot produce their own seed.
2. Hybrid seed production requires considerable technical skill. This makes hybrid seed production a tedius and costly affair.
3. The exploitation of full potential of hybrid varieties requires an adequate supply of irrigation water and fertilizer, and control of weeds, diseases and insect pests. Many farmers are unable to ensure a timely application of these essential inputs.
4. The large scale production of hybrid seed depends on easy emasculation of the female parent, and on an adequate pollen dispersal from the male parent. In many species, emasculation and pollen dispersal are either unsatisfactory or not practically feasible. In such species, *e.g.*, in most self-pollinated crops hybrid varieties cannot be produced on a commercial scale unless their floral biology is adequately modified through breeding/genetic manipulations.
5. In most cross-pollinated species, the requirements of isolation are rigid and, ordinarily, difficult to fulfil, except on large farms. This renders hybrid seed production rather difficult in a country like India where farm holdings are generally small.
6. The amount of hybrid seed required to cover the entire area under any crop appears to be impossible to produce with our present seed production setup. At present, the total certified seed production in the country is just enough to cover only about 4.6% of the total seed requirement of all the crops (including self-pollinated crops, where seed production is relatively much easier).

Achievements through Hybrid Varieties

Hybrid varieties have been commercially exploited in most of the cross-pollinated crops. The greatest use of hybrid varieties has

been made in maize. Other cross-pollinated crop species where hybrid varieties have been commercially exploited are jowar, bajra, sugarbeets (*B. vulgaris*), sunflower (*H. annuus*), broad bean (*Vicia faba*), onions (*A. cepa*), etc. Hybrid varieties have been successful in some self-pollinated crops as well, *e.g.*, cotton (*Gossypium sp.*) in India, tomatoes in Europe and rice in China. Most of the self-pollinated crops do show heterosis, but hybrid varieties are not feasible due to the difficulties in hybrid seed production.

Hybrid varieties were the first to exploit heterosis in maize in this country. The first hybrid varieties in maize were released in 1961 when four hybrids, *viz.*, Ganga 1, Ganga 101, Ranjit and Deccan, were released for cultivation. All these hybrids are double crosses. Subsequently, several other hybrids were released. At present, about a dozen double cross hybrid varieties are recommended for cultivation. These hybrids include Ganga 3, Ganga 4, Ganga 5, Ganga 7, Deccan 101, Hi-Starch and Ganga Safed 2. These hybrids give 25-40 per cent higher yields than the local open-pollinated varieties. The hybrids have yielded up to 4 tonnes per hectare of grain on the farmer's fields.

Hybrid varieties have been commercially used in bajra. The first hybrid bajra was developed by the Punjab Agriculture University,

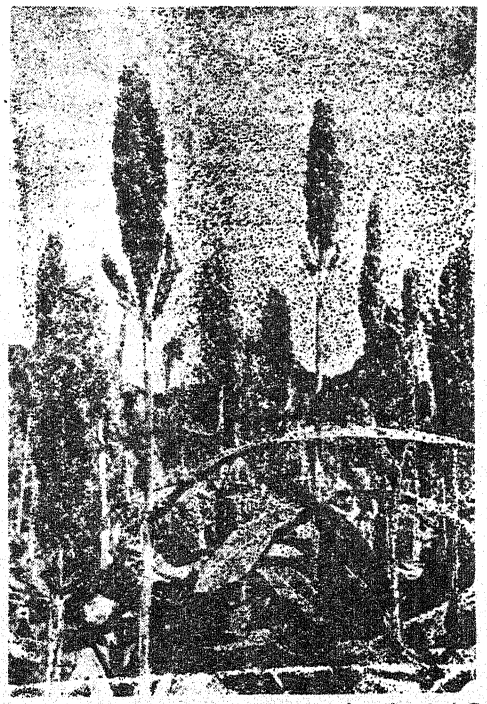


Fig. 19.4. A close view of a hybrid Jowar (*Sorghum bicolar*) Crop (CSH-*).

Ludhiana, and was released in 1965 as HB 1. Subsequently, two other hybrids, HB 3 and HB 5, were released. These hybrids gave yields up to 7 tonnes per hectare in the National Demonstration Trials. However, these hybrids were highly susceptible to downy mildew and ergot due to the high susceptibility of the female parent Tift 23A. These bajra hybrids are single crosses produced by using cytoplasmic-genetic male sterility ; Tift 23A introduced from Tifton, Georgia (U.S.A.), was used as the female parent.

Backcrossing and mutation breeding was used to develop several male sterile lines of bajra resistant to downy mildew and ergot. These male sterile lines are MS 521, MS 541A, MS 570A, MS 5071 and L 111A. Four hybrid bajra varieties based on the new male sterile lines have been released for cultivation. These hybrids are, PHB 10, PHB 11, BJ 104 and BK 560, which are resistant to downy mildew and are recommended for general cultivation in the bajra growing areas. These hybrids are capable of yields up to 6 tonnes per hectare under optimum management.

Hybrid varieties have been released for commercial cultivation in jowar and cotton (Chapter 16). Hybrid vigour has been exploited in many clonal crops and in several fruit trees. An excellent example is provided by coconut (*Cocos nucifera*). The open-pollinated variety West Coast Tall yields upto 110 nuts per plant per year

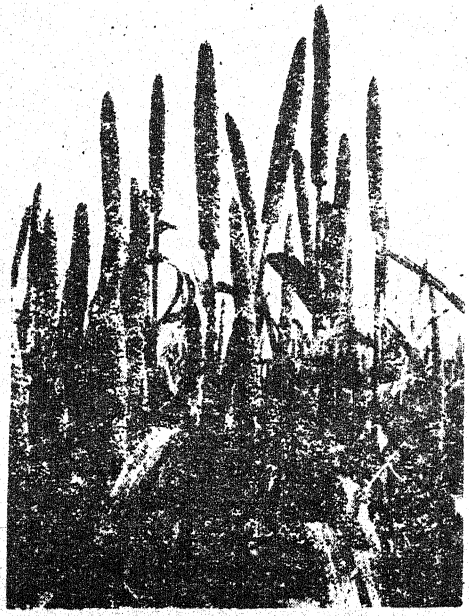


Fig. 19.5. A close view of a hybrid bajra (*Pennisetum americanun*) crop (HB-4)

under optimum management. The hybrid between West Coast Tall and Chowghat Dwarf Orange (T♀ × D♂ for short) yields upto 175 nuts per plant, while the reciprocal hybrid (D♀ × T♂) yields upto 200 nuts per plant. The coconut example also provides evidence for cytoplamic effect on hybrid vigour (compare the performances of the T × D and D × T hybrids). In addition to being higher yielding, the T × D and D × T hybrids are earlier in first flowering (earlier by about 10-11 months) than the parents.

Synthetic Varieties

The possibility of commercial utilization of synthetic varieties in maize was first suggested by Hayes and Garber in 1919. Synthetic varieties have been of great value in the breeding of those cross-pollinated crops where pollination control is difficult, *e.g*, forage crop species, many clonal crops like cacao, alfalfa (*M. sativa*), clovers (*Trifoulim* sp.) etc. Even in maize improvement, synthetic varieties are becoming increasingly important. The maize improvement programme in India now places a considerable emphasis on synthetic varieties. The maize programme of CIMMYT, Mexico, is based on population improvement ; the end-product of such a programme is usually a synthetic variety. The same applies, to a lesser extent, to the pearl millet (*P. americanum*) improvement programme of ICRISAT, India.

Definitions

A synthetic variety is produced by crossing in all combinations a number of lines that combine well with each other. Once synthesized, a synthetic is maintained by open-pollination in isolation. Some breeders use the term synthetic variety in a restricted sense : a synthetic variety is regularly reconstructed from the parental lines and is not maintained by open-pollination. In this chapter, however, the term is used to describe varieties maintained by open-pollination after their production from a number of lines.

Another term, *composite*, is often used as a synonym for synthetic, which is not entirely accurate. A *composite* variety is produced by mixing the seeds of several phenotypically outstanding lines and encouraging open-pollination to produce crosses in all combinations among the mixed lines. The lines used to produce a composite variety are rarely tested for combining ability with each other. Consequently, the yields of composite varieties cannot be predicted in advance (in constrast to synthetics) for the obvious reason that the yields of all the F_1s among the component lines are not available. Like synthetics, composites are commercial varieties and are maintained by open-pollination in isolation.

Germplasm complexes are produced by mixing seeds from several lines or populations of diverse genetic origin. The objective of germplasm complexes is to serve as reservoirs of germplasm. Germplasm complexes are experimental populations and not commercial varieties.

Operations in Producing A Synthetic Variety

By definition, a synthetic variety consists of all possible crosses among a number of lines that combine well with each other. *The lines that make up a synthetic variety may be inbred lines, clones, open-pollinated varieties, short-term inbred lines or other populations tested for GCA or for combining ability with each other.* The operations involved in the production of synthetic varieties are depicted in Fig. 19.6 and are briefly described below.

Evaluation of Lines for GCA. GCA of the lines to be used as the parents of synthetic varieties is generally estimated by topcross or polycross test. *Polycross* refers to the progeny of a line produced by pollination (usually natural pollination) with a random sample of pollen from a number of selected lines. Polycross test is the most commonly used test in forage crops. Polycross progeny are generally produced by open-pollination in isolation among the selected lines. The lines are evaluated for GCA because synthetic varieties exploit that portion of heterosis which is produced by GCA. The lines that have high GCA are selected as parents of a synthetic variety.

Production of A Synthetic Variety. A synthetic variety may be produced in one of the following two ways :

1. Equal amounts of seeds from the parental lines (Syn_0) are mixed and planted in isolation. Open-pollination is allowed and is expected to produce crosses in all combinations. The seed from this population is harvested in bulk ; the population raised from this seed is the Syn_1 generation.
2. All possible crosses among the selected lines are made in isolation. (The parental lines constitute the Syn_0 generation.) Equal amounts of seed from each cross is composited to produce the synthetic variety. The population derived from this composited seed is known as the Syn_1 generation.

The available experimental evidence suggests that both the above methods produce comparable results.

Multiplication of Synthetic Varieties. After a synthetic variety has been synthesised, it is generally multiplied in isolation for one or more generations before its distribution for cultivation. This is done to produce commercial quantities of seed, and is a common practice in most of the crops, *e.g.*, grasses, clovers (*Trifolium sp.*), maize etc. But in some crops, *e.g.*, sugarbeets (*B. vulgaris*), the synthetic varieties are distributed without seed increase, *i.e.*, in the Syn_1 generation.

The open-pollinated progeny from the Syn_1 generation is termed as Syn_2, that from Syn_2 as Syn_3 etc. The performance of Syn_2 is expected to be lower than that of Syn_1 due to the production of new genotypes and a decrease in heterozygosity as a consequence of random mating. However, there would not be a noticeable decline in the subsequent generations produced by open-pollination (Syn_3, Syn_4, Syn_5 etc.) since the zygotic equilibrium for any gene is reached after

me generation of random mating. The synthetic varieties are usually naintained by open-pollinated seed, and may be further improved hrough population improvement schemes, particularly through ecurrent selection.

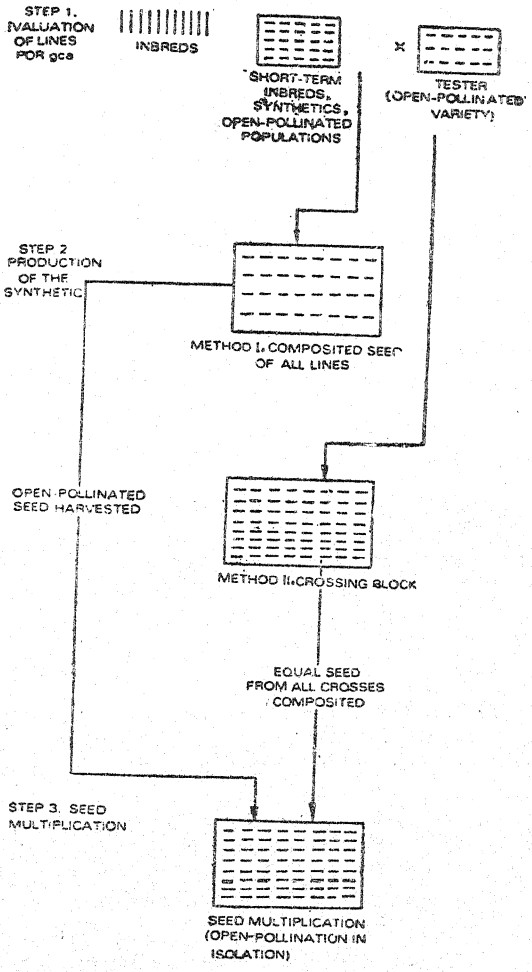


Fig. 19.6. Steps involved in the production of synthetic varieties.

lerits of Synthetic Varieties

Synthetic varieties offer several unique advantages in compa- ion to hybrid varieties in the breeding for heterosis. These advan- ges are listed below.

Synthetic varieties offer a feasible means of utilizing heterosis in crop species where pollination control is difficult. In such species, the production of hybrid varieties would not be commercially viable.

2. The farmer can use the grain produced from a synthetic variety as seed to raise the next crop. If care is taken to avoid contamination by foreign pollen, and to select a sufficiently large number of plants to avoid inbreeding, the synthetics can be maintained for several years from open-pollinated seed. Unlike hybrid varieties, the farmer does not have to purchase new seed every year.
3. In variable environments, synthetics are likely to do better than hybrid varieties. This is so because of the wider genetic base of synthetic varieties in comparison to that of hybrid varieties.
4. The cost of seed in the case of synthetic varieties is relatively lower than that of hybrid varieties. Thus in a country like India, where most of the farmers have limited financial resources, synthetic varieties are more attractive than hybrid varieties.
5. Seed production of hybrid varieties is a more skilled operation than that of synthetic varieties.
6. Synthetic varieties are good reservoirs of genetic variability. The composites and germplasm complexes also serve as gene reservoirs. Germplasm complexes are, in fact, great reservoirs of genes created by man for this purpose alone.
7. There is good evidence that the performance of synthetic varieties can be considerably improved through population improvement without appreciably reducing variability. This offers a possibility for a continuous improvement, which is not possible with hybrid varieties.

Demerits of Synthetic Varieties

1. The performance of synthetic varieties is usually lower than that of the single or double cross hybrids. This is because synthetics exploit only GCA, while the hybrid varieties exploit both GCA and SCA.
2. The performance of synthetics is adversely affected by lines with relatively poorer GCA. Such lines often have to be included to increase the number of lines making up the synthetic as lines with outstanding GCA are limited in number.
3. Synthetics can be produced and maintained only in cross-pollinated crop species, while hybrid varieties can be produced both in self and cross-pollinated crops.

Factors Determining The Performance of Synthetic Varieties

The yield of Syn_2, as noted earlier, is lower than that of Syn_1 due chiefly to production of new gene combinations and, to some extent, a loss in heterozygosity, both being the consequences of random mating in Syn_1. Random mating in Syn_1 leads to a marginal to appreciable loss in heterozygosity in Syn_2 as compared to that in Syn_1. The magnitude of decline in heterozygosity in

Syn_2 depends on the number of inbreds involved in a synthetic and on the proportion of inbreds having different alleles of a given locus. In case of a synthetic constituted by six inbreds, the parental inbreds may represent any one of the seven combinations of genotypes at a single locus (Table 19.1). Combinations I and VII would obviously lead to homozygosity at this locus in Syn_1 and the subsequent generations. Combinations II to VI would produce Syn_1 with 33.3 to 60 per cent heterozygosity at this locus (Table 19.2). The Syn_2 (obtained through random mating in Syn_1) from these combinations, however, would show 27.8 to 50 per cent heterozygosity. Thus there is a decline of 5.5 to 10% in heterozygosity in Syn_2 as compared to that in Syn_1. This decline ranges from 8-12%, 4.1 – 8.2% and 3.1 – 7.1% in the synthetics constituted from five, seven and eight inbreds.

When short-term inbreds are used for the production of synthetic varieties, some of the inbreds would be heterozygous for the gene in question. It can be readily shown that this situation reduces the maximum heterozygosity attainable in Syn_1 as well as the magnitude of decline in heterozygosity in Syn_2. In the case of a synthetic constituted from six short-term inbreds, heterozygosity in Syn_1 may range from 16.7 (one inbred heterozygous, the remaining homozygous for the same allele) to 53.3% (four inbreds heterozygous, one inbred homozygous for one and another homozygous for the other allele). The magnitude of reduction in heterozygosity in Syn_2 may range from 1.4 – 5.8%, which is appreciably lower than the values listed in Table 19.2.

Table 19.1. The different possible combinations of genotypes at a single locus in the six parental inbreds of a synthetic based on six inbreds.

Combination	*Inbred*					
	1	*2*	*3*	*4*	*5*	*6*
I	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
II	*AA*	*AA*	*AA*	*AA*	*AA*	*aa*
III	*AA*	*AA*	*AA*	*AA*	*aa*	*aa*
IV	*AA*	*AA*	*AA*	*aa*	*aa*	*aa*
V	*AA*	*AA*	*aa*	*aa*	*aa*	*aa*
VI	*AA*	*aa*	*aa*	*aa*	*aa*	*aa*
VII	*aa*	*aa*	*aa*	*aa*	*aa*	*aa*

Table 19.2. Heterozygosity (%) in the Syn_1 and Syn_2 generations of a synthetic constructed from the six inbreds listed in Table 19.1

Generation	*Inbred genotype combination*						
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*
Syn_1	0	33.3	53.3	60	53.3	33.3	0
Syn_2	0	27.8	44.4	50	44.4	27.8	0
Decline in heterozygosity in Syn_2	0	5.5	8.9	10	8.9	5.5	0

The yield of Syn_2, as noted earlier, is lower than that of Syn_1 mainly due to the loss in heterozygosity as a result of random mating. The decrease in yielding ability of Syn_2 generation would depend upon (1) the number of lines (Syn_0 populations) constituting the synthetic, and (2) on the difference in the yielding abilities of Syn_1 and Syn_0 generations. *Syn_1 is the first generation synthetic produced by mating in all combinations n parental lines (designated as Syn_0).* This relationship was first suggested by Sewall Wright in 1922 and may be represented as follows.

$$\overline{Syn_2} = \overline{Syn_1} - [(\overline{Syn_1} - \overline{Syn_0}) / n \ ]$$

where, $\overline{Syn_2}$ is the performance of the synthetic variety after one generation of random mating, $\overline{Syn_1}$ is the performance of the synthetic in the first generation after it has been synthesised from the parental lines (it could be the average performance of the all possible single crosses among *n* lines), $\overline{Syn_0}$ is the average performance of the *n* parental lines, and *n* is the number of lines entering into the synthetic variety.

The performance of Syn_3 and the subsequent generations obtained by random mating is expected to be comparable to that of Syn_2, and there should be no further decline. Available evidence shows that the above formula estimates the yield of Syn_2 populations quite reliably. It is apparent from this relationship that the performance of Syn_2 can be improved in 3 different ways : (1) by increasing the number of lines entering into the synthetic, (2) by increasing the performance of Syn_1, and (3) by improving the performence of Syn_0, or the parental lines.

Increasing The Number of Parental Lines. It is clear from the above formula that, theoretically, an increase in the number of lines entering a synthetic would improve the performance of its Syn_2. This improvement would be more noticeable with the smaller values of *n*, *e.g.*, 1/2, 1/3, 1/4, 1/5 etc., but would become less noticeable as the *n* becomes larger, *e.g.*, 1/10, 1/11, 1/12 etc.

Practically, lines with outstanding GCA are few. Inevitably, as the *n* is increased, lines with poorer GCA would have to be included in the synthetic. This reduces the performance of Syn_1 and, therefore, that of Syn_2. Thus *n* cannot be increased beyond a certain level without adversely affecting the performance of Syn_1 unless several lines with outstanding GCA are available. Generally, a compromise has to be made between the two opposing forces. Obviously, the appropriate *n* would depend upon the GCA of the available lines. If several lines with high GCA are available, a larger *n* would be appropriate than when a fewer lines with high GCA are available.

In practice, the number of lines entering a synthetic variety varies from 3-15, but 4-10 is the most common number. Some of the maize composites recommended for commercial cultivation in India have as many as 18 (Composite C_1) or even 22 (Composite Agaiti 76)

parental lines. In contrast, some other composites are crosses between two populations of maize, *e.g.*, composite Tarun is derived from Syn P 200 × Kisan, or are derived from a single population, *e.g.*. Chandan Safed composite is an improved version of Zapolote Chico, an exotic variety, and Makki Safed 1 composite is derived from Bhodipur white open-pollinated variety of maize.

Increasing The Performance of Syn_1. The performance of Syn_1 is the average performance of all single crosses among the parental lines. Clearly, Syn_1 performance depends upon GCA of the parental lines. As noted previously, the Syn_1 performance is limited by the non-avilability of a sufficient number of lines with outstanding GCA. At the same time, if the number of lines is kept too low, say, 3 and 4, Syn_1 performance would be high but the Syn_2 performance would be adversely affected due to the low number of parental lines.

Improving The Performance of Parental Lines (Syn_0). Syn_2 performance can be improved by increasing the performance of parental lines. This can be achieved in the following manner. *First*, second cycle and third cycle inbreds may be developed as their performance would be better than that of the first cycle inbreds. *Second*, inbreds may be isolated after the population has been subjected to recurrent selection for GCA. And *third*, short-term inbred lines or even non-inbred lines or populations may be used for the production of synthetic varieties.

The use of short-term inbreds is an attractive idea. Plants differ in GCA in the early stages of inbreeding process ; even S_0 plants differ in GCA. Thus the synthetic varieties may be constituted from plants or lines that have undergone limited inbreeding, *e.g.*, for one or two generations, or no inbreeding at all. Theoretically, the performance of a Syn_2 from non-inbred lines is expected to be higher than that of a Syn_2 derived from inbred lines if the performance of the Syn_1 populations is comparable or equal. Further, the peak performance of Syn_2 would be attained with a relatively smaller number of noninbred lines than with inbred lines. It may be pointed out that sythetics constituted by crossing of populations subjected to RSGCA and RRS would serve the same purpose as developing synthetic varieties from short-term inbreds or non-inbreds.

Maintenance of Synthetic Varieties

Synthetics are generally maintained from open-pollinated seed produced in isolation. The seed preserved for raising the next generation is a random sample large enough to prevent inbreeding. However, there is evidence that the genetic constitution of synthetic varieties changes due to natural and artificial selections. This may adversely affect the performance of synthetic varieties, but in some cases it may improve the performance appereciably. Hence it is desirable to reconstitute synthetic varieties at regular intervals from the parental lines. Exact reconstitution of a synthetic variety is possible when the parental lines are inbreds or clones. But when the parental

lines are noninbred or open-pollinated populations, an exact reconstitution is not possible since the parental lines themselves are subject to changes in gene frequencies due to the evolutionary forces. In such cases, the synthetic varieties have to be maintained from open-pollinated seed, or have to be reconstituted from the parental lines which might have undergone genetic changes.

The synthetic varieties have considerable genetic variability which responds to population improvement. They offer an excellent opportunity for further improvement in yielding ability through selection, particularly recurrent selection. If care is taken to minimise inbreeding, selection may be expected to produce gains through several cycles of selection.

Test for Combining Ability in Species Where Pollination Control Is Difficult

Synthetic varieties offer a feasible means for exploiting a substantial portion of heterosis in crop species where pollination control is difficult. In such species, usually noninbred populations or clones (where possible) are used for the production of synthetic varieties. The clones or populations that enter in a synthetic must be evaluted for GCA. The technique for testing GCA in case of maize, where pollination control is easy, was considered in connection with hybrid varieties, and is based on topcross progeny test. In this case, the topcross seed results from inbred × open-pollinated variety cross. But in species where pollination control is difficult, topcross seed also includes seed from selfing (except where the clones are self-incompatible) and from intercrossing with other selected clones or populations. Consequently, several techniques for testing GCA have been developed for use in such species.

Open-Pollinated Progeny Test. Open-pollinated progenies are derived from seed produced by random mating of selected plants or clones with other plants or clones present in the nursery. Random mating takes place by the natural forces that favour cross-pollinationin the species in question, and no pollination control is exercised. Thus open-pollinated seed also contains some selfed seed. Open-pollinated progeny may be similar to polycross progeny when only the selected clones are planted in the crossing block.

Topcross Test. Topcross seed is obtained by crossing the selected clones with an open-pollinated variety. In practice, it also contains selfed seed and seed produced by outcrossing with the other selected clones. The proportion of topcross seed can be increased by planting a greater number of plants of the tester strain.

Polycross Test. Polycross test is based on the seed obtained by random mating of a selected clone with all the other selected clones. To facilitate random meeting among the clones, each clone is planted at several locations in the nursery, preferably at different dates. Polycrosses are generally not perfect, *i.e.*, mating is nonrandom, and in many cases the mating may be highly nonrandom.

These three tests measure the GCA of selected clones or populations. Tests for SCA are based on the single-cross test as in the case of maize.

Single-Cross Test: The *n* selected clones are crossed with each other to produce $n(n-1)/2$ possible single crosses. This is done in one of two ways : *first*, each pair of clones to be crossed is planted in isolation, and *second*, inflorescences from each pair of clones are enclosed in separate bags. The single-cross test provides an estimate of SCA of the specific cross combinations, and also of GCA of the clones.

In practice, polycross test is the most commonly used for estimating GCA in such crop species. The available evidence indicates that it is a more reliable test for GCA than open-pollinated progeny test or top-cross test.

Achievements through Synthetic Varieties

Synthetic varieties have been widely used in forage crops and in crops where pollination control is difficult. The maize breeding programme at CIMMYT, Mexico and the pearl millet breeding programme at ICRISAT, Hyderabad (to some extent) are based on synthetic varieties generally developed through population improvement. The maize breeding programme in India is placing increasingly greater emphasis on the production of synthetic or composite varieties.

In India, the first composite varieties were released in 1967 ; the six maize composites were, Ambar, Jawahar, Kisan, Vikram, Sona and Vijay. Subsequently, a number of other composites have been released by both the central and provincial variety release committees. At present, there are about 25 maize composites recommended for cultivation in the various parts of country. These composites range from those based on local materials (Makki Safed 1 and Moti), through those involving both Indian and exotic sources (Mansar, Chandan 3, Diara Composite etc.) to those developed from exotic lines only (Chandan Safed 2, Amber Pop, Chandan 1 etc.). Some of the composites involve as many as 18 (composite C_1) or even 22 (Agaity 76) lines, while certain others involve only two (Jawahar, Kisan, Sona) or even one (Chandan Safed 2, A-de-Cuba) populations.

Some of the recently released maize composites are : Co 1 (full season, resistant to downy mildew), NLD (full season, white seeded), Renuka (very early), Kanchan (very early), and Diara 3 (developed by three cycles of full-sib selection in Diara Composite).

These composities yield as much as 90 per cent of the best hybrid varieties. Three *opaque 2* composites, *viz* , Shakti, Ratan and Protina, have been released. They have twice the amount of lysine and tryptophan as compared to the normal maize hybrids and composites. Thus the nutritional value of these composites is superior to the other composites and hybrids.

SUMMARY

The two methods of breeding that utilize heterosis are hybrid and synthetic varieties. *Hybrid varieties* are the F_1 generations from crosses between two clones, inbreds or other populations. Commercial hybrid varieties are either single crosses or double crosses. Single cross hybrid varieties are common in bajra, jowar, cotton, rice (in China) and maize (in U.S.A). Double cross varieties are common in maize in India. The production of hybrid varieties involves the following steps : development of inbreds by close inbreeding, selfing or from haploid plants present in natural populations in low frequencies (less than 10^{-5}) ; evaluation of inbreds, first by phenotypic evaluation, followed by topcross test for GCA and single cross test for SCA and for prediction of double cross performance ; and production of hybrid seed using genetic male sterility, *e.g.*, castor in U.S.A., cytoplasmic male sterility, cytoplasmic-genetic male sterility, *e.g.*, maize, jowar, bajra, sunflower, self-incompatibility, *e.g.*, *Brassica* species or manual emasculation and/or pollination, *e.g.*, cotton in India, tomatoes in Europe.

Inbreds may be tested for GCA early in the inbreeding programme ; it is known as *early testing*. Selection for GCA during inbreeding is also effective. Inbreds may be improved by pedigree selection, backcross method, convergent improvement and gamete selection. Backcross method and pedigree selection have been widely used Gamete selection is a promising scheme and should find a greater use in the breeding of hybrid varieties in future.

Synthetic varieties are produced by crossing in all possible combinations a number of lines that combine well with each other and are maintained by open-pollination in isolation. The lines that serve as parents of synthetic varieties may be clones, inbreds, synthetic or other populations. The steps in production of synthetic varieties are : evaluation of parental lines for GCA using topcross or polycross test ; production of synthetic varieties by mixing the seeds of all parental lines and harvesting open-pollinated seed from the resulting crop or by making all possible single crosses and mixing their seed ; and multiplication of the seed thus produced.

Synthetic varieties offer many advantages over hybrid varieties : they are practically feasible means of exploiting heterosis in species where pollination control is difficult ; seed production is simpler and cheaper ; farmer can save his own seed ; they serve as germ plasm reservoirs and they may be expected to perform better than hybrids in a variable environment. However, synthetics exploit only the GCA portion of heterosis and cannot utilize that part of heterosis produced due to SCA.

The performance of synthetics may be improved in the following ways : by increasing the number of parental lines, but this cannot be done beyond a certain point wihtout adversely affecting the performance ; by increasing the performance of Syn_1 generation, which depends on the GCA of the parental lines ; and by increasing the performance of the parental lines which may be easily done by using short-term inbreds or noninbreds as parents.

In species where pollination control is difficult, combining ability may be estimated by open-pollinated progeny test, topcross test, polycross test or single cross test. Polycross test is the most commonly used. The seed of synthetic varieties is maintained by open-pollination in isolation, but they may be reconstituted from parental lines at regular intervals.

QUESTIONS

1. Define the following : hybrid varieties, genetic emasculation, single cross, testcross, topcross, double cross, tester, detasselling, population cross, synthetic varieties, composite varieties, germplasm complexes.
2. Give a brief historical account of the development of hybrid varieties with special reference to India.
3. Describe briefly the various operations for the production of hybrid varieties.

4. Describe the various methods of hybrid seed production.
5. Discuss in detail the evaluation of inbreds and the prediction of double cross performance on the basis of data on performance of single crosses.
6. Discuss the uses of self-incompatibility and male sterility in hybrid seed production. Briefly describe the production of new cytoplasmic male sterile lines.
7. List the various methods of improvement of inbred lines. Describe any two of them in detail and discuss their usefulness.
8. Discuss the achievements through hybrid varieties in cross and self-pollinated species with a special reference to the Indian experience.
9. Write short notes on the following : (i) prediction of double cross performance, (ii) use of self-incompatibility in hybrid seed production, (iii) hybrid varieties, (iv) synthetic varieties, (v) order of inbreds in a double cross, (vi) second cycle inbfeds, and (vii) test for GCA in species where pollination control is difficult.
10. Briefly describe the various operations in production of synthetic varieties. How are synthetic varieties maintained ? Briefly describe the achievements through synthetic varieties.
11. Briefly discuss the effects of various factors on the performance of synthetic varieties. What measures would you suggest for the improvement of the performance of synthetic varieties.
12. Discuss in detail the merits and demerits of synthetic varieties as compared to the hybrid varieties.

Suggested Further Reading

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CHAPTER 20

Clonal Selection And Hybridization

Some agricultural crops and a large number of horticultural crops are asexually propagated. Some common asexually progagated crops are sugarcane (*S. officinarum*), potato (*S. tuberosum*), sweet potato (*I. batatas*), *Colocasia* (Taro), *Arum*, *Dioscorea* (yams), *Mentha*, ginger (*Zingiber sp.*), turmeric (*C. domestica*), banana (*Musa paradisiaca*) etc., almost all the fruit trees, *e.g.*, mango (*Mangifera indica*), citrus (*Citrus sp.*), apples (*P. malus*), pears (*P. communis*), peaches (*P. persica*), litchi (*Litchi chinensis*), loquat (*Eriobotrya japonica*) etc., and many ornamentals and grasses. Many of these crops show reduced flowering and seed set, *e.g.*, sugarcane, potato, sweet potato, banana etc., and some varieties of these crops do not flower at all. But many of these crops flower regularly and show satisfactory seed set. However, they are propagated asexually to avoid the effects of segregation and recombination, both being the inevitable consequences of sexual reproduction. Segregation and recombination produce new gene combinations due to which the progeny differ from their parents in genotype and phenotype. Asexual reproduction, on the other hand, produces progeny exactly identical to their parents in genotype because the progeny are derived from vegetative cells through mitosis. *The advantage of asexual reproduction is immediately clear : it preserves the genotype of an individual indefinitely. It must be noted that this does not depend on the homozygosity of the genotype of an individual. Any genotype is preserved and maintained through asexual reproduction. In contrast, self-pollination preserves and maintains* only *homozygous genotypes giving rise to purelines.*

Characteristics of Asexually Propagated Crops

Asexually propagated crops generally have the following characteristics.

1. A great majority of them are perennials, *e.g.*, sugarcane, fruit trees etc. The annual crops are mostly tuber crops, *e.g.*, potato, cassava (*M. utilissima*), sweet potato etc.

2. Many of them show reduced flowering and seed set. Many varieties do not flower at all. Only the crops grown for fruit, particularly where good fruit set depends upon seed formation, show regular flowering and satisfactory seed set.
3. They are invariably cross-pollinated.
4. These crops are highly heterozygous and show severe inbreeding depression.
5. A vast majority of asexually propagated crops are either polyploids, *e.g.*, sugarcane, potato, sweet potato etc., or have polyploid species or varieties.
6. Many species are interspecific hybrids, *e.g.*, banana (*M. paradisiaca*), sugarcane, *Rubus* etc.
7. These crops consist of a large number of *clones*, that is, progeny derived from a single plant through asexual reproduction. Thus each variety of an asexually propagated crop is a clone.

Clone

A clone is a group of plants produced from a single plant through asexual reproduction. Thus asexually propagated crops consist of a large number of clones. Therefore, these crops are also known as clonal crops. All the members of a clone have the same genotype as the parent plant. As a result, they are identical with each other in genotype. Consequently, the phenotypic differences within a clone do not have a genetic basis and are purely due to the environmental effects. A selection within a clone is thus useless. The various characteristics of a clone are summarised below.

1. *All the individuals belonging to a single clone are identical in genotype.* This is so because a clone is obtained through asexual reproduction which involves mitotic cell division only. Genetic variation in the progeny of a plant is produced chiefly by segregation and recombination which occur during meiosis only. Thus the genotype of a clone is maintained indefinitely without any change.
2. *The phenotypic variation within a clone is due to the environment only.* This is so because all the individuals belonging to a single clone have the same genotype.
3. *The phenotype of a clone is due to the effects of genotype (G), the environment (E) and the genotype×environment interaction (G×E) over the population mean* (μ). The phenotype (P) of a clone may be expressed as $P=\mu+G+E+GE$. Thus the phenotypic differences among clones would be partly due to their genotype and partly due to E and GE components. Hence the efficiency of selection among clones, as among purelines, would depend upon the precision with which the E and GE components of phenotype are estimated (Chapter 4).
4. *Theoretically, clones are immortal, i.e.*, a clone can be maintained indefinitely through asexual reproduction. But clones

usually degenerate due to viral or bacterial infections. A clone may become extinct due to its susceptibility to diseases or insect pests. Further, genetic variation may arise within a clone changing its characteristics.

5. Generally, clones are highly heterozygous and show severe loss in vigour due to inbreeding.

Genetic Variation within a Clone

Genetic variation within a clone may arise due to somatic mutation, mechanical mixture and occasional sexual reproduction.

Mutation. Somatic mutations are also known as bud mutations. The frequency of mutations is generally very low (10^{-5}—10^{-7}). Ordinarily,only dominant mutations would be expressed in the somatic tissues because recessive mutations can be expressed only in the homozygous condition. A mutant allele would be homozygous only when (1) both the alleles in a cell mutate at the same time producing the same mutant allele, or (2) the mutant allele is already in the heterozygous condition in the original clone. Both these events are possible, but they are expected to be rare. Therefore, we may conclude that dominant bud mutations express themselves more frequently than recessive ones. Another aspect of bud mutations is that they often produce chimeras, *i.e.*, individuals containing cells of two or more genotypes. However, it is not a great problem because normal plants, *i.e.*, nonchimeras, may be produced from chimeras through one of several techniques.

Bud mutations make possible the selection of buds to establish new desirable clones, the process being known as *bud selection*. Bud selection is of some importance in the improvement of perennial crops like fruit trees, or of those crops where flowering does not take place. It requires a large number of plants to be observed and several trained persons to detect the mutant buds. Thus bud selections are generally practised in commercial plantations and not in the breeder's nurseries. Further, the discovery of desirable bud mutations has been more of an accident than a result of planned search for them.

Mechanical Mixture. Mechanical mixture produces genetic variation within a clone much in the same manner as in the case of purelines.

Sexual Reproduction. Occasional sexual reproduction would lead to segregation and recombination. The seedlings obtained from sexual reproduction would, therefore, be genotypically different from the asexual progeny.

It is evident that old clones would tend to become variable, at least in the annuals and biennials, *e.g.*, potato. A selection within an old clone may be useful in establishing a new and more desirable clone. For example, Kufri Red potato is a selection from Darjeeling Red Round ; it was derived from a single disease-free plant. Similarly, Kufri Safed potato is a selection from Phulwa.

Comparison among Clones, Purelines and Inbreds

The various characteristics of clones, purelines and inbreds are summarised in table 20.1. It is clear that plants within a clone, pureline or inbred have the same genotype although clones are heterozygous, while purelines and inbreds are homozygous or nearly so. Clones are maintained by asexual reproduction, but purelines and inbreds are maintained by self-pollination or close inbreeding.

Table 20.1. Comparison among clones, purelines and inbreds.

Particulars	*Clone*	*Pureline*	*Inbred*
Mode of pollination in the crop species where they occur	Cross-pollination	Cross-pollination	Cross-pollination
Natural mode of reproduction in such species	Asexual (in most cases)	Sexual	Sexual
Genetic make-up of the plants in natural populations of such species	Heterozygous	Homozygous	Heterozygous
Obtained through	Asexual reproduction from a single plant	Natural self-pollination from a single homozygous plant	Artificial self-pollination (or other forms of inbreeding) and selection for several generations
Maintained through	Asexual reproduction	Natural self-pollination	Artificial self-pollination or close inbreeding
All the plants in a single entity are genotypically	Identical	Identical	Almost identical
Used directly as a variety	Yes	Yes	No (Used in developing hybrid or synthetic varieties)
The genetic make-up of plants within a variety	Heterozygous	Homozygous	Almost homozygous
Organism where found	Plants	Plants	Plants, animals

Clonal Degeneration

Theoretically, clones are immortal. In other words, clones can be maintained indefinitely. But in practice, clones have a limited life and often older clones lose vigour and productivity. *The loss in vigour and productivity of clones with time is known as clonal degeneration.* The term clonal degeneration implies that the degeneration is inherent in the clone, *i.e.*, it is due to the vegetative reproduction itself. But there is no evidence that such is the case. Detailed studies show that clonal degeneration results from (1) mutation, (2) viral diseases and (3) bacterial diseases.

Mutation. Mutation is a recurrent process, Therefore, it may become a problem over a long period of time. Further, some mutations occur at a relatively high frequency. For example, 'bolter' mutations

in patato occur at a frequency of 10^{-3} in some clones, and are a serious problem in the maintenance of these clones.

Viral Diseases. Viral disease are easily transmitted through vegetatative propagules. Therefore, they easily spread with time in the case of clonal crops. Viruses are perhaps responsible for more cases of clonal degeneration than any other single cause. Many clones in potato have been lost due to virus infections.

Bacterial Diseases. Some cases of clonal degeneration are the results of bacterial infections. The ratoon stunting disease of sugarcane (*S. officinarum*) is caused by a small bacterium. There are other similar instances where a bacterium is responsible for the degeneration of clones.

Another reason for the deterioration of clones may be somatic mutations but they are not likely to be the common cause. The vegetative reproduction itself does not affect the vigour or the productivity of clones. On the other hand, asexual reproduction keeps intact the specific gene combinations which make a clone superior to or more desirable than other clones. It is likely that with further investigations more and more cases of clonal degeneration would be found to be the results of bacterial and viral infections.

Methods of Improvement of Asexually Propagated Crops

Asexually propagated crops differ from the sexually propagated ones in one basic aspect that the breeding materials and the commercial varieties are maintained by asexual reproduction in the case of the former. This provides some unique advantages and opportunities in their breeding. *A single outstanding plant selected from a population forms the basis of a new variety. The breeding behaviour or genotype of the plant is not important since there would be no further sexual reproduction.* The outstanding plant may be selected from an old unimproved variety, an improved variety that has become variable, or from a population produced by crossing two or more clones. Thus the breeding of clonal crops has two well defined phases which are the same as those in the case of sexually reproducing crops. These phases are : *first*, the utilization of already existing variability, and the creation of variability through hybridization where it does not exist, and *second*, selection of the best genotype to produce a superior clone or variety. *The procedure of selection is known as clonal selection since the selected plants are used to produce new clones.*

We shall first examine the main features of clonal selection and later discuss the procedure for creation of variability through hybridization.

Clonal Selection

The phenotypic value of a plant or a clone is due to the effects of its genotype (G), the environment (E) and the genotype × environment interaction (GE). Of these, only the G effcets are heritable

and, therefore, stable. The environmental and interaction effects are nonheritable and cannot be selected for. Therefore, a selection for quantitative characters based on the observations on single plants is highly unreliable. In fact, plants selected in this way may be no better than a random sample. Further, a selection for characters like yielding ability etc. on the basis of unreplicated clonal plots would often be misleading and unreliable. The value of a clone can be reliably estimated only through replicated yield trials. However, selection for highly heritable characteristics, such as, plant height, days to flowering, colour, disease resistance etc., are easy and effective even on the basis of individual plants or single plots. Clearly, these situations are the same as those in the case of sexually reproducing crops.

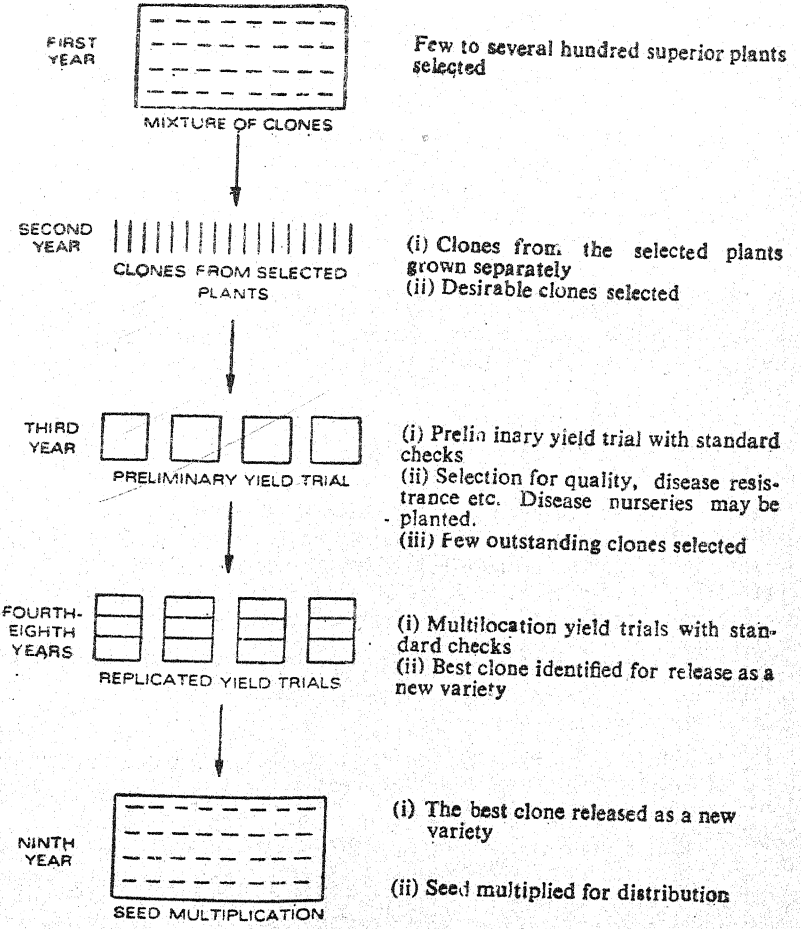


Fig. 20.1. A generalised scheme for clonal selection in asexually propagated species.

In view of these considerations, in the earlier stages of clonal selection, when selection is based on single plants or single plots, the emphasis is on the elimination of weak and undesirable plants or clones. The breeder cannot reasonably hope to identify superior genotypes at this stage. In the later stages when replicated trials are the basis of selection, the emphasis is to identify and select the superior clones. The various steps involved in clonal selection are briefly described below and are depicted in Figure 20.1.

First Year. From a mixed variable population, few hundred to few thousand desirable plants are selected. A rigid selection can be done for simply inherited characters with high heritability. Plants with obvious weaknesses are eliminated.

Second Year. Clones from the selected plants are grown separately, generally without replication. This is because of the limited supply of propagating material for each clone, and because of the large number of clones involved.

The characteristics of clones will be more clear now than in the previous generation when the observations were based on individual plants. The number of clones is drastically reduced, and inferior clones are eliminated. The selection is based on visual observations and on the breeder's judgement of the value of clones. Fifty to one hundred clones are selected on the basis of clonal characteristics.

Third Year. Replicated preliminary yield trial is conducted. A suitable check is included for comparison. Few superior performing clones with desirable characteristics are selected for multilocation trials.

At this stage, selection for quality is done. If necessary, separate disease nurseries may be planted to evaluate disease resistance of the selected clones.

Fourth to Eighth Years. Replicated yield trials are conducted at several locations alongwith a suitable check. The yielding ability, quality and disease resistance etc. of the clones are rigidly evaluated. The best clones that are superior to the check in one or more characteristics are identified for release as varieties.

Ninth Year. The superior clones are multiplied and released as varieties.

Merits of Clonal Selection

1. It is the only method of selection applicable to clonal crops. It avoids inbreeding depression, and preserves the gene combinations present in the clones.
2. Clonal selection, without any substantial modification, can be combined with hybridization to generate the necessary variability for selection.
3. The selection scheme is useful in maintaining the purity of clones.

Demerits of Clonal Selection

1. This selection method utilizes the natural variability already present in the population ; it is not devised to generate variability.
2. Sexual reproduction is necessary for the creation of variability through hybridization.

Hybridization

Clonal crops are generally improved by crossing two or more desirable clones, followed by selection in the F_1 progeny and in the subsequent clonal generations. Once the F_1 has been produced, the breeding procedure is essentially the same as clonal selection. The improvement through hybridization involves three steps : (1) selection of parents, (2) production of F_1 progeny, and (3) selection of superior clones.

Selection of Parents. Selection of parents to be used in hybridization is very important since the value of F_1 progeny would depend upon the parents used. Parents are generally selected on the basis of their known performance both as varieties and as parents in hybridization programmes. The performance of a strain in hybridization programmes depends on its prepotency and general combining ability. It would be highly desirable to know the relative values of GGA and SCA in the crop to be improved. If GCA is more important, a small number of parents with good GCA should be used in hybridization programmes. On the other hand, when SCA is more important, a large number of parents should be used to produce a large number of F_1 families.

A recent suggestion is to partially inbreed the parents to be used in hybridization programmes. Clonal crops show severe inbreeding depression, but it is expected that one generation of selfing or 2-3 generations of sib mating may not reduce vigour and fertility too severely. Inbreeding may enable the breeder to identify plants that would have a greater concentration of desirable genes. These plants may be more prepotent as parents than the highly heterozygous clones. This practice is gaining some favour with plant breeders.

Production of F_1 Progeny. Generally, clonal crops are cross-pollinated and they may show self-incompatibility. The selected parents may be used to produce single crosses involving two parents or an equivalent of a polycross involving more than two parents.

Selection among F_1 Families. When the breeding value of the parents is not known, and the relative contribution of GCA and SCA is not available, a large number of crosses have to be made in order to ensure that atleast some of the crosses would produce outstanding progeny in F_2. This is particularly true in a species where crop improvement has not been done or has been done at a small scale. In such cases, it would be cumbersome to evaluate a large number of F_1 progeny in detail. To avoid this, generally small samples of several

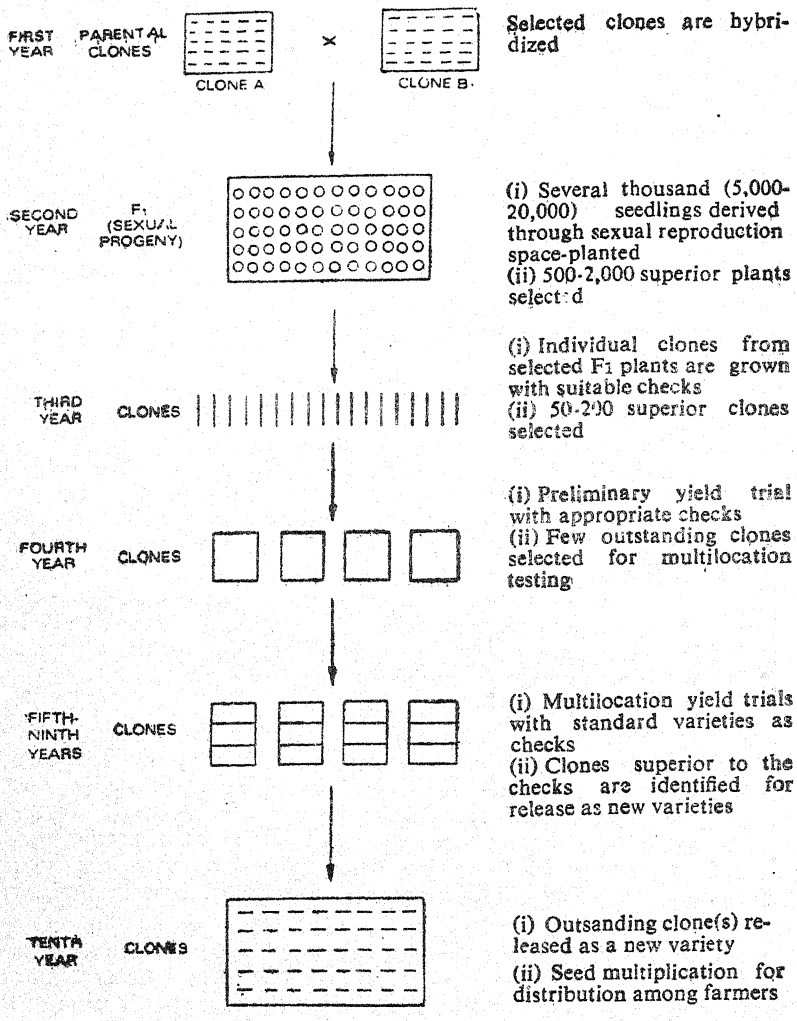


Fig. 20.2. A generalised scheme for breeding of annual asexually propagated species through hybridization

F_1 populations are grown. The general worth of individual F_1 families or populations is estimated visually. The presence of outstanding individuals in these families is also noted. Inferior families are eliminated. Promising families with outstanding individuals are then grown at a much larger scale for selection. The procedure is designed to save time, space and labour by planting only small populations of a large number of crosses at the preliminary stage.

Selection within F_1 Families. The selection procedure within F_1 families is essentially the same as that in the case of clonal selection. The various steps involved in the breeding of clonal crops through hybridization are listed below (Fig. 20.2). From step 2 onward, these should be read alongwith the steps in clonal selection.

First Year. Clones to be used as parents are grown and crosses are made to produce F_1 progeny.

Second Year. Sexual progeny from the cross, *i.e.*, seedlings obtained from seeds, are grown. Undesirable plants are eliminated. Few hundred to few thousand desirable plants are selected.

Third Year. Clones from individual plants are grown separately. Poor and inferior clones are eliminated. Upto 200 superior clones may be selected for preliminary yield trial.

Fourth Year. A replicated preliminary yield trial is conducted. A suitable check is included for comparison. Few outstanding clones are selected for trials at several locations.

Fifth to Ninth Year. Replicated yield trials are conducted at several locations. A suitable check is included for comparison. One or a few outstanding clones are identified and released as new varieties.

Tenth Year. The clones released as varieties are multiplied and distributed among farmers.

Interspecific Hybridization in the Improvement of Clonal Crops

Interspecific hybridization has been successfully used in the improvement of clonal crops. Many varieties of *Rubus*, *Malus*, strawberries etc. are interspecific hybrids. Potato and sugarcane, two of the clonal crops that concern us the most, have also benefited from interspecific hybridization. Potato variety Kufri Kuber was developed from a complex cross (*Solanum curtilobum* × *Solanum tuberosum*) × *Solanum andigena*. This variety shows much less clonal degeneration in the plains than the variety Up to-date. Generally, interspecific crosses are made to transfer specific characters, such as, disease resistance, from the wild species to the cultivated potato, *S. tuberosum*. For example, *Solanum demissum* has been extensively used as a source of late blight resistance.

Sugarcane is a special case. All the sugarcane varieties now in cultivation have been developed from complex crosses between *Saccharum officinarum*, *S. barberi*, *S. robustum* and *S. spontaneum*. *S. spontaneum* has been used to combine its hardiness and disease

resistance with high sugar content and high yielding ability of *S. officinarum*. These noblised canes give higher yields of both cane and sugar and have higher sugar content than the noble canes themselves. Similarly, interspecific hybridization has been a useful tool in the improvement of other clonal crops. The principal reason for such a great success of interspecific hybridization in the clonal crops is their asexual reproduction ; this completely avoids segregation and recombination. Another reason is that most of them are not seed crops, hence flowering and fertility are not essential for their success as varieties.

Problems in the Breeding of Asexually Propagated Crops

It may appear that the breeding of asexually propagated crops is simple, but in actual fact it is not. There are several problems peculiar to clonal crops which are difficult to resolve. There are three major problems in their breeding : (1) reduced flowering and fertility, (2) difficulties in genetic analysis, and (3) perennial life cycle.

Reduced Flowering and Fertility. Clonal crops grown for vegetative parts generally show reduced flowering ; some varieties do not flower at all, *e.g.*, sugarcane, potato, sweet potato, *Colocasia* etc. These crops also show considerable sterility due to polyploidy (potato, sugarcane, sweet potato etc.), complex interspecific hybridity and cytoplasmic male sterility. The reduced flowering and high sterility seem to be a result of correlated responses to selection for increased yields of vegetative parts.

In fruit crops, flowering does occur, but seed set is generally much reduced. The possible causes for reduced seed set are polyploidy (pears, *Rubus*, some apples, bananas, strawberries etc.) and interspecific hybridity (*Rubus*, strawberries, bananas etc.). In addition, parthenocarpy occurs in many fruit trees, *e.g.*, pineapple, *Citrus*, fig etc. Facultative apomixis occurs in mango and *Citrus*, which is a nuisance when the breeder desires to obtain sexual progeny from crosses for further selection. *Because of reduced flowering and seed set, many desired clones cannot be used in hybridization programmes of many clonal crops.*

Difficulties in Genetic Analyses. As discussed earlier, estimates of GCA and SCA are very helpful in the selection of suitable parents for hybridization programmes. Genetic analysis in the most of clonal crops is very difficult due to one or more of the following : (1) reduced flowering, (2) sterility and (3) perennial life cycle.

Perennial Life Cycle. Perennial life cycle drastically increases the time required for obtaining sexual progeny for the genetic analysis of clones. Genetic analysis in most of the fruit trees is generally not attempted for this reason. Replicated yield trials, so familiar in the case of annuals, are not possible in the case of perennials.

Achievements

Clonal Selection. Several varieties of clonal crops have been developed either by bud selection or by clonal selection from heterogeneous clones. Kufri Red potato is a clonal selection from Darjeeling Red Round ; it was developed from a single disease-free plant. Similarly, Kufri Safed is a clonal selection from the potato variety Phulwa. Bud selection has been done in several fruit trees. For example, Bombay Green banana is considered to be a bud selection from Dwarf Cavendish ; Pidi Monthan from Monthan, and High Gate from Gross Michel.

Hybridization. A large number of varieties of clonal crops have been developed through hybridization. Some prominent potato varieties developed through hybridization are Kufri Alankar, Kufri Kuber, Kufri Sindhuri, Kufri Kundan, Kufri Chamatkar, Kufri Chandramukhi etc. Kufri Jyoti is late blight resistant and Kufri Sheetman is

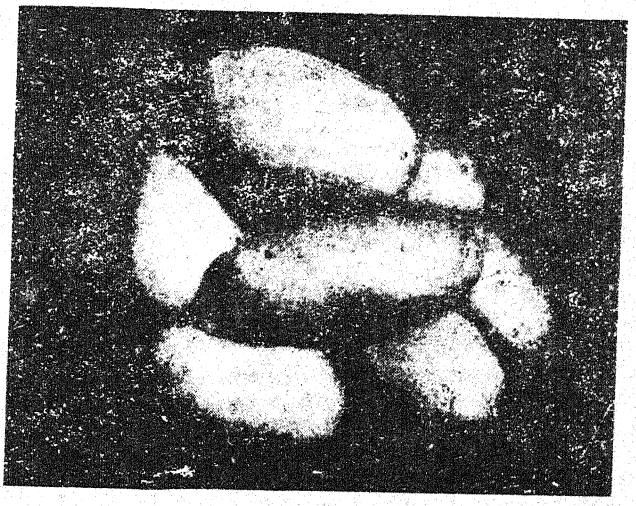


Fig. 20.3. Tubers of 'Kufri Chandramukhi', an early maturing variety of potato developed through hybridization.

frost resistant. In case of sugarcane, all the varieties have been developed through interspecific hybridization. Some prominent varieties of sugarcane are CO 1148, CO 1158, COS 510, CO 975, COS 109, CO 541 etc.

SUMMARY

Many agricultural and a large number of horticultural crops are asexually propagated. The varieties in such crops are *clones i.e.*, asexual progeny of single plants. Clonal crops are highly heterozygous and show severe inbreeding depression. They are cross-pollinated, perennial, usually polyploid, many are interspecific hybrids and show reduced flowering and fertility.

Clones are homogeneous, stable and theoretically immortal. Genetic variation within a clone may arise by mechanical mixture, mutation or sexual reproduction. Clones may degenerate due to infection by bacteria and viruses, and possibly due to mutation. Clonal crops are improved by *clonal selection* within a clone showing variation or by *hybridization* followed by clonal selection. Interspecific hybridization has been extensively used for the improvement of several clonal crops. Clonal selection essentially consists of selection of superior plants to produce new improved clones. Clonal field crops, such as potato and sugarcane, have been improved by clonal selection as well as hybridization.

QUESTIONS

1. What is a clone ? What are the main characteristics of clones ? Compare clones, inbreds and purelines.
2. Discuss the various sources of genetic variation within clones. How would you create variation in a clonal crop ?
3. What is clonal selection ? Describe the method for clonal selection. Discuss its merits, demerits and achievements.
4. What are the various steps involved in the improvement of clonal crops through hybridization ? Describe these steps in brief. Discuss the problems and achievements of hybridization in the improvement of the clonal crops.
5. With the help of suitable examples, describe the contribution of interspecific hybridization in the improvement of asexually reproducing species.
6. Discuss in detail the salient features of improvement programmes in the following clonal crops : (i) potato, (ii) sugarcane.
7. Explain clonal degeneration. Does clonal degeneration have a genetic basis ? Discuss the above in the light of the factors responsible for clonal degeneration.

Suggested Further Reading

FERWERDA, F.P. and WIT, F. (eds.) 1969. Outlines of Perennial Crop Breeding in the Tropics, Veeman and Zonen, Wageningen, The Netherlands.

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CHAPTER 21

Breeding for Disease Resistance

Disease is an abnormal condition in the plant produced by an organism. The plant affected by a disease is known as *host*, while the organism that produces the disease is termed as *pathogen*, Clearly, abnormalities produced by nonbiological environment or by the genetic factors present in the host are not diseases. Diseases are produced by a variety of organisms from both plant and animal kingdoms, *viz.*, fungi, bacteria, viruses, nematodes and insects. Different crops are attacked to different degrees by the different kinds of pathogens, but it may be emphasised that all the crop species are attacked by them. For example, cereals suffer from epidemics of air-borne fungi, most solanaceous crops are infected severely by viruses, cotton is damaged by many insects and so on. In the order of their importance (based on the damage caused by them), the pathogens may be listed as fungi>bacteria>viruses> nematodes=insects. Much of the breeding effort has been directed against diseases caused by fungi, which may be greater than the effort against all the other pathogens put together. Therefore, our discussion would be primarily based on the knowledge of fungal diseases. Breeding for insect resistance is dealt with in some detail in the next chapter (Chapter 22).

LOSSES DUE TO DISEASES

Diseases reduce total biomass (dry matter) production by the crop in one or more of the following ways : killing of plants, killing of branches, general stunting, damage to the leaf tissues and damage to the reproductive organs including fruits and seeds. The degree of damage would depend upon the intensity of infection but both need not be proportional. Satisfactory scales are available for monitoring the intensity of infection, but there is no scale available for assessing the economic loss caused by a disease. However, there is no doubt that diseases reduce biomass and consequently yield. Often the loss due to diseases may range from a few to 20 or 30 per cent ; in cases of severe infection, the total crop may be lost. A striking effect of diseases is the disappearance from cultivation of otherwise excellent but susceptible varieties. Wheat

(*T. aestivum*) variety Kalyan Sona dominated the Indian agriculture for about a decade, but had to be abandoned as it became susceptible to leaf rust. Similarly, another wheat variety Janak was eliminated from cultivation due to its susceptibility to Karnal bunt. Another feature of diseases is that for each crop species, the importance of different diseases keeps on changing. Diseases that were minor in the past become important due to the changes in crop varieties and agricultural practices, *e.g.*, *Helminthosporium* leaf blight in maize (USA), and leaf hoppers and tungro virus in rice (India). New diseases may also be introduced alongwith plant materials introduced from other countries ; quarantine measures are directed against such movements of diseases and insect pests (Chapter 2).

HISTORY OF BREEDING FOR DISEASE RESISTANCE

Theophrastus, in the third century B.C., noted that cultivated varieties differed in their ability to avoid diseases. That diseases are produced by a pathogen was conclusively shown by Benedict Prevost ; he showed that wheat bunt was produced by a fungus. During the middle of nineteenth century, various workers, *e.g.*, Andrew Knight, noted that crop varieties differed for disease resistance. In 1905, Biffen demonstrated that resistance to yellow rust in wheat was governed by a recessive gene segregating in the ratio 3 : 1 in F_2. Subsequently, in 1904, Blakeslee described mating-type differentiation in *Rhizopus*.

In 1894, Erikson showed that pathogens, although morphologically similar, differed from each other in their ability to attack different related host species. Later, in 1911, Barrus showed that different isolates of a microorganism differed in their ability to attack different varieties of the same host species ; this finding is the basis for physiological races and/or pathotypes. It was subsequently established that the ability of a pathogen to infect a host strain, *i.e.*, *pathogenicity*, is genetically determined. Thus both the ability of the host to resist invasion by a pathogen as well as the ability of a pathogen to invade its host are genetically controlled. In 1955, Flor postulated the hypothesis of gene-for-gene relationship between host and pathogen, which holds true in most of the cases and is widely accepted.

The information available so far clearly shows that the disease resistance of host is not a function of the host genotype alone, it is also determined by the genotype of the pathogen as well. The host strains differ in resistance, while those of the pathogen differ in pathogenicity ; both variations have genetic basis. The pathogen has a remarkable capacity for generating new variation in pathogenicity by a variety of reproduction methods and mutation. Thus the task of breeders is not only to develop varieties resistant to the prevalent pathotypes of the pathogens, but also to be ready to face the challenge to be posed by the new pathotypes in future.

MECHANISMS FOR THE GENERATION OF VARIABILITY IN PATHOGENS

Pathogens have a wide range of flexible mechanisms for producing variability. The following discussion is based on fungi, but many of the features and the conclusions drawn are applicable to

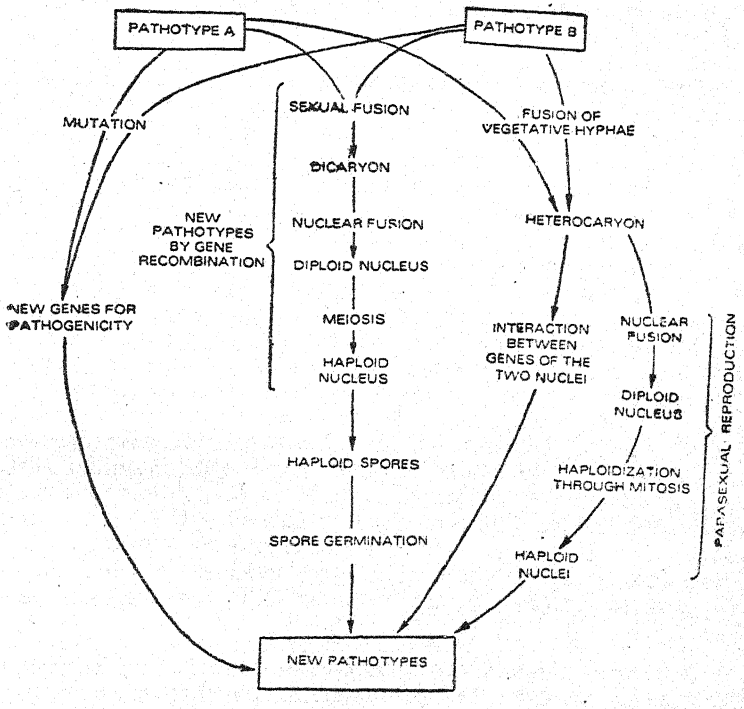


Fig. 21.1. The possible pathways for production of new pathotypes in pathogenic fungi.

other pathogens as well. New variability in fungal pathogens may be created in the following ways : (1) mutation, (2) sexual reproduction, (3) heterokaryosis, and (4) parasexual reproduction (Fig. 21.1).

Mutation. Spontaneous mutations are the ultimate source of all genetic variation present. Spontaneous mutations occur at a low rate of 10^{-5} to 10^{-6}. But this rate is large enough when it is considered alongwith the astronomical number of asexual and sexual spores produced by fungi.

Sexual Reproduction. Sexual reproduction is a common feature of most of the pathogenic fungi, but in the pathogens belonging to Fungi Imperfecti sexual reproduction has not been detected. Sexual reproduction involves the fusion of two haploid cells, which may or may not be sexually differentiated, to produce a cell containing

two nuclei, a *dicaryon.* The duration of dicaryon stage varies from a very brief period (Phycomycetes and primitive Ascomycetes) to the entire life cycle, except for brief diploid and haploid generations (smuts). *The two nuclei of a dicaryon ultimately fuse to produce a diploid nucleus which undergoes meiosis to produce four haploid nuclei.* The haploid nuclei may or may not divide mitotically before they produce haploid sexual spores, which on germination give rise to the haploid phase of life cycle.

The two cells that fuse to produce a dicaryon may belong to the same hypha ; such a fungus species is known as *homothallic.* In *heterothallic* fungi, the fusing cells must be of two different mating types, hence from two different hyphae. There are two mating types, + and −, which are generally controlled by a single gene with two alleles, mt^+ and mt^-. But in some cases, two or more genes are involved and in certain other cases multiple alleles are known. Mating type is not associated with sexual differentiation ; the latter is controlled by certain other genes. The same mating type often produces both the male and female gametes, but fusion would occur between the gametes from different mating types only. *Thus the mating type in fungi is not sexual differentiation, but it is similar to self-incompatibility genes of higher plants. Heterothallism,* thus ensures the fusion of genetically dissimilar nuclei to produce the dicaryon, while *homothallism* permits the fusion of cells from the same hypha. But in the case of homothallism as well, dissimilar nuclei may fuse. In general, homothallism appears to be less efficient in the generation of variability than heterothallism. But homothalism is common in fungi and is known in all the groups of fungi. This obvious success of homothallism is a good evidence for the ability of the system to generate the necessary variability for adaptation and survival.

The fusion of two haploid, genetically dissimilar nuclei, followed by meiosis, produces new gene combinations through segregation and recombination. Sexual reproduction is the most common means of recombination of the existing variability. A less frequent, but equally potent, means of genetic recombination is parasexual reproduction in fungi.

Heterocaryosis. In many fungi, *e.g.*, Fungi Imperfecti, the hyphae are multinucleate during the active growth. These nuclei may be genetically identical (*Homocaryosis*) or of dissimilar genotypes (*heterocaryosis*). Heterocaryosis is produced by the fusion of vegetative hyphae which are genetically dissimilar ; this fusion is not affected by the mating type. Heterocaryosis occurs in nature, and is of adaptive value. Heterocaryons are generally more vigorous than homocaryons. During mitosis, the different nuclei of a heterocaryon may get included in different cells producing homocaryons. Thus heterocaryosis is capable of storing a limited amount of genetic variability which is released through 'segregation' of whole nuclei during

mitosis ; this process appears to have an adaptive significance. There is evidence that heterocaryosis can change the pathogenicity and may even give rise to new races of a pathogen.

Parasexual Reproduction. *In parasexual reproduction, diploid nuclei are produced in vegetative cells from which haploid nuclei are produced through mitotic irregularities, but not through meiosis.* Diploid cells occur in the heterocaryons at a frequency of 10^{-7} as a result of the fusion of two nuclei present in a heterocaryon. These diploid cells give rise to haploid cells at a frequency of 10^{-8}. Crossing over does not take place during haploidization of diploid nuclei ; hence a chromosome is inherited as a single unit. However, somatic recombination does take place in a low frequency, *i.e.*, one chiasma pier 10 nuclei. But somatic recombination is not associated with soma tc reduction (production of haploid nuclei through mitosis) ; the two events are independent of each other and may take place in the same or in different nuclei.

Thus heterocaryosis may produce a different race of the pathogen through an interaction among the genes two nuclei. But when it is coupled with parasexual reproduction, it produces new pathogenic races through recombination of entire chromosomes ; occasionally there may be a recombination of genes as well (when somatic recombination has occurred before somatic reduction).

PHYSIOLOGICAL RACES AND PATHOTYPES

The concept of physiological races was introduced by Barrus in 1911. *Physiological races* are strains of a single pathogen species differing in their ability to attack different varieties of the same host species. The varieties of a host species used to identify physiological races of a pathogen are known as *differential host* or *host testers.* Differential hosts are chosen on the basis of differences in their resistance to the pathogen, but the genes for resistance present in them are usually not known. Ideally, each of the differential hosts should possess a single resistance gene different from those present in the others. If this were so, n differential hosts would be able to identify 2^n physiological races if there were only two types of host-pathogen reaction, *e,g.*, resistant and susceptible. If, however, more types of reaction were identifiable, as is the case in stem rust of wheat, a still larger number of races can be identified ; the number of races identifiable would be r^n, where, r=number of reaction types identifiable, and n=number of differential hosts with a single unique resistance gene. Identification of physiological races can be explained using bunt (a fungal disease) resistance of Martin and Turkey wheat varieties (Table 21.1). A set of 18 differential hosts, each carrying a single gene is available for linseed stem rust, and a set of 12 differentials is available for late blight in potatoes. How many races can these differentials identify assuming two types of reaction ?

A pathogen race capable of attacking a host strain carrying a

those unable to attack this strain are known as *avirulent*. When the strains of a pathogen are classified on the basis of their virulence to known resistance genes present in the host, they are referred to as *pathotypes* or *biotypes*. The ideal differentials, each carrying a single distinct resistance gene, would identify pathotypes (Table 21.2).

Table 21.1. The identification of 4 physiological races of *Tilletia* sp. producing wheat bunt using two differential wheat varieties.

Differential Host	*Reaction to bunt*			
	Race I	*Race II*	*Race III*	*Race IV*
Martin	R*	R	S*	S
Turkey	R	S	R	S

*R=resistant, and S=susceptible

Pathotype classification is more precise than that of the physiological races. It is because in the case of physiological races the resistance genes present in the differential hosts are generally not known, and a differential host may possess two or more resistance genes. There is a growing trend to change to pathotype classification from physiological race classification. *It may be pointed out that pathotype classification is limited by the availability of new resistance genes and by the evolution of new pathotypes virulent to the hitherto resistant genes.*

It would be seen that physiological races are in fact pathotypes if each of the differential hosts carry a single and distinct resistance gene. But if some of the differentials carry two or more resistance

Table 21.2. Pathotype classification based on a set of 12 differential hosts, each carrying a single distinct resistance gene (R_1-R_{12}) to *Phytophthora infestans*, the fungus causing potato late blight

Resistance gene present in the differential host	*Pathotype of potato late blight fungus*											
	(P_1)	(P_2)	(P_3)	(P_4)	(P_5)	(P_6)	(P_7)	(P_8)	(P_9)	(P_{10})	(P_{11})	(P_{12})
R_1	S											
R_2		S										
R_3			S									
R_4				S								
R_5					S							
R_6						S						
R_7							S					
R_8								S				
R_9									S			
R_{10}										S		
R_{11}											S	
R_{12}												S

Note : Only the susceptible reactions are shown. Complex pathotypes carrying more than one virulence gene, *e.g.*, $P_{(1,2)}$ or $P_{(1, 5, 8, 12)}$, are identified by their virulence against the respective host genes for resistance (*e.g.*, R_1, R_2 and R_1, R_5, R_8, R_{12}, respectively). A susceptible reaction of the host indicates the virulence of pathotype, while a resistant reaction shows avirulence.

genes, a physiological race may consist of more than one pathotype. Pathotype differentiation is common in air-borne fungi and viruses. Some pathotype differentiation occurs in soil-borne fungi, nematodes and insects.

GENETICS OF PATHOGENICITY

Pathogenicity is the ability of a pathogen to attack a host and is synonymous to virulence. A study of the inheritance of virulence is possible only when a host with a resistance gene is available. Extensive studies have shown that the virulence of a pathogen is under genetic control. More than one gene are generally involved in the control of virulence and at each locus there are usually two alleles. In many pathogens, *e.g.*, rusts and smuts, where dicaryons have a rather longer life, it has been shown that virulence is recessive to avirulence. Why the same has not been demonstrated in pathogens with a brief dicaryotic stage in their life cycles ? (Remember that in such cases, the parasitic stage would be haploid.) With an increasing knowledge of the host-pathogen relationship, it is customary to think about virulence in terms of specific resistance genes present in the host and not in terms of host varieties that may have one or more genes for resistance.

The inheritance of pathogenicity is explained using the example of *Venturia inaequalis*, the Ascomycete that causes apple scab disease. Apple variety Wealthy is resistant to some strains (avirulent) of the pathogen and susceptible to others (virulent). A cross between an avirulent and a virulent strain produces two types of ascospores, virulent and avirulent, in 1 : 1 ratio indicating a single gene control with two alleles. The inclusion of other varieties differing in resistance provides more information on the inheritance of pathogenicity. Some strains of *Venturia* are virulent towards Wealthy but are avirulent towards McIntosh variety of apple, while some others show just the opposite pathogenicity. When two such strains are crossed, the resulting ascospores are of 4 types : virulent on both Wealthy and McIntosh, virulent on Wealthy, virulent on McIntosh, and avirulent on both (Table 20.3). This clearly shows the involvement of two genes in the control of pathogenicity on Wealthy and McIntosh. With more resistant hosts, a finer analysis of pathogenicity may be done in terms of the number of genes governing pathogenicity. In case of late blight potato, 12 different genes are known to govern virulence toward the corresponding resistance genes present in the host.

DISEASE DEVELOPMENT

The development of diseases produced by fungi occurs in four well defined stages : contact, infection, establishment and development. *Contact* represents the landing of a pathogen on the host tissue. *Infection* is the process by which the pathogen gains entry

Table 21.3. The inheritance of pathogenicity in the ascomycete *Venturia inaequalis* causing apple-scab. The notation of strain *A* and *B* is arbitrary. Note that when only one resistant variety of the host is used, pathogenicity appears to be governed by a single gene. But when two different resistant varieties are used, there is evidence for two genes. More genes may be detected by using more varieties differing in their resistance genes.

Pathogen strain	*Host variety*		*Remarks*
	Wealthy	*McIntosh*	
A	*R**	*S**	
B	*S*	*R*	
A×*B* (ascospores tested on Wealthy)	*R* (50% of ascospores) *S* (50% of ascospores)		Ratio 1 : 1, conclusion : one gene with 2 alleles
A×*B* (ascospores tested on McIntosh)	(50% of ascospores) (50% of ascospores)	*R* *S*	Ratio 1 : 1, conclusion : one gene with two alleles
A×*B* (ascospores tested on both)	*R* (25% of ascospores) *R* (25% of ascospores) *S* (25% of ascospores) *S* (25% of ascospores)	*R* *S* *R* *S*	Approximate ratio 1 : 1 : 1 : 1, two genes each with two alleles

*S**=susceptible, and *R*=resistant. Susceptible reaction of the host denotes virulence of the pathogen, while resistant reaction indicates avirulence.

into the host tissue. Usually, spores germinate and enter the host tissue through natural openings, such as, stomata, or through wounds. But many pathogens can enter the host tissue through the epidermis by producing some hydrolytic enzymes. *Both contact and infection stages are greatly affected by the environment and provide the means for disease escape.* But once the pathogen has entered the host tissue, the *establishment* phase begins. In this phase, the pathogen proliferates and spreads within the host tissue, but the symptoms of the disease are not visible during this stage. The next phase, the *development phase,* is characterised by the development of characteristic symptoms of the disease and is generally associated with spore production (or multiplication). *The rate of spore production or multiplication is the crucial factor in the spread of diseases as the spores serve as inoculum for the uninfected plants.* Usually, disease resistance in a host involves a restriction on the establishment and, particularly, multiplication. In most cases of disease resistance, infection and a certain degree of establishment do take place ; the resistance usually reduces the degree of establishment and the rate of spore production.

DISEASE ESCAPE

As the name suggests, *disease escape* refers to the freedom of susceptible host varieties from a disease due purely to the environmental

factors. Disease escape occurs primarily by avoiding contact, but unfavourable weather conditions may prevent infection. Disease escape may be a result of the environmental factors, early varieties, changed date of planting, change in the site of planting, use of resistant root stocks, balanced application of NPK, control of the disease carrier and control of the pathogen itself. The disease development requires a suitable environment for spore germination and infection ; an *unfavourable environment* would reduce or prevent the germination of spores and infection. For example, susceptible varieties of potato (*S. tuberosum*) escape late blight attacks in years when the growing period is relatively dry. *Early varieties* often escape a disease since they mature before the disease epidemic occurs, *e.g.*, early groundnut varieties generally escape 'tikka' disease and early potato varieties may escape late blight. *A changed date of planning* may successfully avoid a pathogen. Virus-free seed potato is produced by sowing the crop in October instead of November, the normal planting time in Jullundur and some other places. The October-planted crop escapes aphid infestation and, consequently, virus infection since during October-January the aphid population is very low in these places. In case of soil-borne fungi, *e.g.*, root rots, wilts etc., and other soil-borne diseases, it is highly effective to *change the site of planting*, *Resistant root stocks* to soil-borne diseases have been successfully used in many fruit trees to grow otherwise susceptible, but desirable, varieties. *A balanced application of NPK* is of some usefulness as potassium is known to enhance the ability of a host to resist diseases, while N is known to increase succulence and susceptibility. Some diseases, particularly viral diseases, are transmitted by insects ; the *control of the insect vector* in such cases is extremely useful in controlling the disease. The control of yellow mosaic in mung (*V. radiata*) is based on the control of white fly, the vector for the yellow mosaic virus. In the end, it may be pointed out that *chemical disease control* directed against the pathogen is useful in protecting susceptible varieties, but it is costly and generally pollutes the environment.

DISEASE RESISTANCE

Host varieties are classified as susceptible or resistant according to their response to the pathogen. The various reactions of the hosts to the various pathogens may be grouped into the following types : susceptible, immune, resistant and tolerant. In case of some diseases, the host strains either show or do not show the disease, hence the classification of disease reaction is simple. But in most cases, the host reaction is not so sharply defined and shows a graded variation. Such reactions or infections are classified according to an arbitrary, but practically sound, scale in which the disease score may vary from 0 (with no disease) to 5 or 9 (most susceptible).

Susceptible Reaction. In the case of susceptible reaction, disease development is profuse and is presumably not checked by the

genotype of host. In practice, the susceptible reaction is classifiable in relative terms only, that is, in relation to the reaction of other host varieties available and the prevailing environment. Wheat variety Agra Local is generally taken as the susceptible standard in scoring wheat rusts.

Immune Reaction. When the host does not show the symptoms of a disease, it is known as immune reaction. Immunity may result from the prevention of pathogen to reach the appropriate parts of the host, *e.g.*, exclusion of the spores of ovary infecting fungi by closed flowering habit of wheat and barley. But more generally, it is produced by hypersensitive reaction of the host usually immediately after the infection has occurred. In hypersensitive reaction, a group of host cells around point of infection die. This severely restricts the establishment of pathogen and eliminates its reproduction. Thus in immune reaction, the rate of reproductions of the pathogen is zero, that is, $r=0$.

Resistance. Resistance denotes a less disease development than in the susceptible variety and is a relative attribute. Infection and establishment do take place, but growth of the pathogen in the host tissue is restricted. This results in smaller spots or pustules than in the susceptible variety. Generally, the rate of reproduction is considerably reduced which limits the spread of disease. *In case of resistance, disease symptoms do develop and the rate of reproduction is never zero, i.e., $r>0$, but it is sufficiently lower than 1 (the rate of reproduction on the susceptible variety) to be useful.* The inhibition of the growth of pathogens is believed to be nutritional in nature, and in some cases chemical growth inhibitors may be involved.

Tolerance. Tolerance implies that the host is attacked by the pathogen in the same manner as the susceptible variety, but there is little or no loss in biomass production or yield. In certain situations this may be so, but often this term is used without sufficient evidence. It is desirable not to use this word unless it has been clearly shown to be the case. Generally, tolerance is difficult to measure since it is confounded with partial resistance and disease escape. To estimate tolerance, the loss in yield or some other trait of several host varieties having the same amount of disease, *e.g.*, leaf area covered by the disease etc., is compared. An example of true tolerance is known in the barely variety Proctor to powdery mildew ; it shows a 13% yield loss as compared to 20% loss in the varieties Zephyr and Sultan. Virologists use the term tolerance to express a lack of symptoms even in the presence of the virus. The lack of symptoms, however, may be strain-specific, and it does not necessarily mean a tolerance in terms of damage caused by the disease. Plant breeders inevitably select for tolerance whenever yield is the basis for selection.

VERTICAL AND HORIZONTAL RESISTANCE

The terms vertical and horizontal resistance were introduced by Van der Plank and are widely used.

Vertical Resistance. It is also known as race-specific, pathotype-specific or simply specific resistance. *Vertical resistance is generally determined by major genes and is characterised by pathotype-specificity.* Pathotype-specificity denotes that the host carrying a gene for vertical resistance is attacked by only that pathotype which is virulent towards that resistance gene ; to all other pathotypes the host will be resistant. Thus an avirulent pathotype will produce an immune response, *i.e.*, $r=0$ or close to 0, but the virulent pathotype will lead to the susceptible reaction, that is, $r=1$ (Table 21.4). *Clearly, immune or susceptible response in the case of vertical resistance depends on the presence of virulent pathotype. When the virulent pathotype becomes frequent, epidemics are common in the case of vertical resistance.*

Table 21.4. The reaction of vertical resistance genes and horizontal resistance polygenes to three pathotypes of potato late blight. Note that the major genes produce resistance to the avirulent pathotypes only, while horizontal resistance produces resistance reaction to all the pathotypes.

Resistance gene	*Pathotype*			*Remarks*
	$P_{(1)}$	$P_{(2)}$	$P_{(3)}$	
Pathotype-specific oligogenes				
R_1	S* (1)**	I* (0)	I (0)	
R_2	I (0)	S (1)	I (0)	Vertical Resistance
R_3	I (0)	I (0)	S (1)	
Pathotype-nonspecific polygenes				
	R (<1)	R (<1)	R (<1)	Horizontal resistance

*S=Susceptible, I=immune, and R=resistant

**figures within parenthesis represent the reproduction rate of pathogen, that is, $r=0$, represents immune response ; $r=1$, denotes susceptible response ; while $r<1$, expressses resistance response.

Horizontal Resistance. It has a number of synonyms, *e.g.*, race-nonspecific, pathotype-nonspecific, and partial or general resistance. *Horizontal resistance is generally controlled by polygenes, that is, many genes with small effects, and it is pathotype-nonspecific* (Table 21.4). That is why it is also known as 'general resistance'. In the case of horizontal resistance, reproduction rate of the pathogen is not zero, but it is less than one, *i.e.*, $r>0$ but <1. Horizontal resistance, therefore, does not prevent the development of symptoms of the disease, but it slows down the rate of spread of the disease in the population.

There is some evidence that polygenes governing horizontal resistance operate on gene-for-gene basis in a way similar to the oligogenes of vertical resistance (VR), but often this is not evident

due to the small individual and interaction (resistance × virulence) effects. More often than not, it is very difficult to separate HR from VR. Further, HR may not always be more stable than VR. In view of these, Parlevliet argues that 'if so-called HR is difficult to separate from VR, and if it is not more stable, perhaps the effort to distinguish between these two types is meaningless'. Several hypotheses have been advanced to account for HR ; these are listed below.

1. *Ghost Gene Hypothesis.* According to this hypothesis, HR represents the cumulative effect of VR genes which have succumbed to the corresponding virulence genes of the pathogen, *i.e.*, 'ghost' or 'residual' effect of VR genes. There is some evidence that such may be the case, but such an effect is always race-specific.

2. HR may be due to the modifying effects of the genetic background on VR genes, and due to several VR genes being present together. This view is similar to the ghost gene hypothesis.

3. HR is governed by polygenes. Oligogenes act as regulators of the HR resistance by making the action of polygenes race-specific.

4. The polygenes show gene-for-gene relationship with minor virulence genes of the pathogen, which means that they are race-specific. But their effect appears to be race-nonspecific HR due to the small interaction effects, and due to a relatively large error variance.

Opinions on the relative values of horizontal and vertical resistance in crop improvement vary considerably. Their usefulness is largely dependent on the crop species and the particular disease in question. In case of potato leaf blight, veritical resistance is regarded as a nuisance, and breeders primarily work for improving horizontal resistance. But in the case of wheat rusts, vertical resistance is the only form of resistance being exploited by breeders. It may be pointed out that horizontal resistance is becoming increasingly important (*e.g.*, in the case of leaf rust of wheat) and is likely to play a greater role in crop improvement in the future.

MECHANISM OF DISEASE RESISTANCE

A variety of mechanisms are involved in disease resistance. In some cases, the basis of resistance is better known than in other cases. The various mechanisms of disease resistance are : (1) mechanical, (2) hypersensitivity, (3) antibiosis, and (4) nutritional.

Mechanical. Certain mechanical and/or anatomical features of the host may prevent infection. For example, closed flowering habit of wheat and barley prevents infection by the spores of ovary infecting fungi.

Hypersensitivity. In a large number of cases, immune reaction is due to the hypersensitivity reaction of the host. Immediately after infection, several host cells surrounding the point of infection die. This leads to the death of the pathogen or at least prevents its spore production. The molecular basis of recognition of the pathogen by the host is not known. It is believed that phytoalexins are responsible for hypersensitive reaction. Phytoalexins are specific polyphenolic or terpenoid chemicals and are produced by the host in response to the infection by a pathogen or even in response to fungi nonpathogenic to the host in question. More than thirty different phytoalexins have been identified. They are present in many plant species, particularly in the members of Leguminosae. Phytoalexins are either fungicidal or fungistatic. This mechanism is found in the case of biotrophic pathogens.

Antibiosis. It has been suggested that the resistance in several cases, particularly to insects, is due to the presence of some toxic substances. But generally critical evidence is lacking and the toxic substances have not been identified. In cotton, cultivars lacking gossypol, the polyphenol normally present in the host species, are more susceptible to boll worm and other insect pests, except the boll weevil to which they are more resistant than the normal varieties.

Nutritional. The reduction in growth and in spore production is generally supposed to be due to unfavourable physiological conditions within the host. Most likely, a resistant host does not fulfil the nutritional requirements of the pathogen and thereby limits its growth and reproduction. However, a more precise information is not available on this aspect.

GENETIC BASIS OF DISEASE RESISTANCE

The first study on genetics of disease resistance was that by Biffen in 1905. He reported the inheritance of resistance to leaf rust of wheat variety Rivet in crosses with some susceptible varieties. In F_2, there were 3 susceptible to 1 resistant plants, indicating that the resistance was controlled by a single recessive gene. Subsequently, several other studies showed that resistances to various diseases are monogenically determined, but cases of duplicate, complementary and other interactions have been reported. Most of the earlier studies were conducted without taking into consideration the physiological specialization (pathotype differentiation) of the pathogen, which can materially influence the conclusions drawn. It is now recognised that disease resistance may show three different modes of inheritance : oligogenic, potygenic and cytoplasmic.

Oligogenic Inheritance

In such cases, disease resistance is governed by one or few major genes, and resistance is generally dominant to the susceptible reaction. The action of major resistance genes may be altered by modifying genes in many cases, *e.g.*, bunt resistance in wheat. But in some other cases, modifying genes are not known, *e.g.*, resistance

to Y and X viruses in potato. Oligogenes generally produce immune reaction. The chief characteristic of the oligogenic disease resistance is its pathotype-specificity, that is, the resistance gene is effective against some pathotypes, while it is ineffective against the others. In most cases, there are a number of major genes that determine resistance to a particular disease, *e.g.*, more than 20 different resistance genes are known for leaf rust of wheat, while those for stem rust resistance exceed 30. The genetics of oligogenic resistance has advanced by two events occurring repeatedly. These events are : (1) the discovery of a resistance gene to the prevalent pathotype, and (2) the evolution of a pathotype virulent to this new resistance gene. *Oligogenic resistance is synonymous to vertical resistance.*

The inheritance of disease resistance may be explained with the help of bean (*P. vulgaris*) anthracnose resistance. There are two physiological races, Alpha and Beta, of the pathogen ; Alpha is virulent on the bean variety Robust but not on White Marrow, while Beta is virulent on White Marrow but not on Robust. When a cross between White Marrow and Robust is tested against the Alpha pathotype, F_1 is resistant and in F_2 a 3 : 1 segregation is observed for resistance and susceptibility. Similar results are obtained when the same cross is tested against the Beta pathotype. Thus resistance of each of the two bean varieties is governed by a single gene which appear to be different. This can be demonstrated by testing the F_2 from the above cross against a pathotype (P_0) which is avirulent on both the varieties. The F_2 shows a 15 : 1 ratio indicating duplicate gene action. A complementary gene action (F_2, 9 : 7 ratio) would be obtained if the F_2 were inoculated with a mixture of the Alpha and Beta races (Table 21.5). *It is evident that the conclusions drawn from inheritance studies on disease resistance are largely determined by the quality of the pathogen inoculum (the pathotype and the purity of the pathotype).*

Gene-for-Gene Relationship. The gene-for-gene relationship between a host and its pathogen was postulated by Flor in 1956 based on his work on linseed (*Linum usitatissimum*) rust. Subsequent studies have shown that the gene-for-gene relationship holds true in most of the cases studied extensively, and is now widely accepted. It has been found that for every resistance gene present in the host, the pathogen has a gene for virulence. Susceptible reaction would result only when the pathogen is able to match all the resistance genes present in the host with appropriate virulence genes. If one or more resistance genes are not matched by the pathogen with the appropriate virulence genes, resistance reaction is the result. In most of the pathogens, virulence is recessive to avirulence.

The gene-for-gene relationship may be explained with the help of linseed rust resistance. Resistance is governed by a number of

Table 21.5. The inheritance of reistance to anthracnose in rajma (*Phassolus vulgaris* L.)

Pathotype	*Rajma variety*			*Ratio in F_2*	
	White Marrow	*Robust*	*White Marrow × Robust (F_1)*	*Resistant*	*Susceptible*
	AAbb	*aaBB*	*AaBb*		
Alpha (P_b)**	*R*	*S*	*R*	3	1
Beta (P_a)	*S*	*R*	*R*	3	1
Avirulent (P_o)	*R*	*R*	*R*	15	1
Alpha + Beta ($P_a + P_b$)	*S*	*S*	*R*	9	7

*Letters within parenthesis denote the genotype of the host.

**$P_{(a)}$, $P_{(b)}$ and $P_{(o)}$ denote the pathotype with reference to the resistance genes *A* and *B*. $P_{(a)}$ is virulent toward *A*, $P_{(b)}$ toward *B* and $P_{(o)}$ toward neither *A* nor *B*.

Note : The conclusions regarding the inheritance of resistence are greatly affected by the inoculum of the pathogen : incoculum $P_{(a)}$ or $P_{(b)}$—F_2 ratio 3 : 1, conclusion—single gene with two alleles ; inoculum $P_{(o)}$—F_2 ratio : 15 : 1, conclusion—duplicate gene action ; inoculum $P_{(a)} + P_{(b)}$ —F_2 ratio 9 : 7, conclusion—complementary gene action.

dominant genes, *e.g.*, *N* and *P*. Virulence of the pathogen is determined by recessive genes for virulence designated as *a*. A subscript denoting the resistance gene toward which *a* is virulent is also added. The resistance determined by the host gene *NN* would be overcome only by the pathotype carrying the virulence gene $a_N a_N$; all other pathotypes will not be able to attack the host. Similarly, resistance due to gene *PP* can be overcome by the pathotype with gene $a_P a_P$ only, and that due to genes *NN PP* by the pathotype $a_N a_N$ $a_P a_P$ only (Table 21.6).

When the host carries more than one gene for resistance, the pathogen produces a pathotype that has virulence genes for all the resistance genes. For example, in potato (*S. tuberosum*) 12 resistance genes (R_1, R_2,.....R_{12}) are known for late blight. A pathotype virulent toward all the 12 genes has been known. But there are some cases, where the pathogen has not been able to produce a matching virulence gene for the resistance gene. For example, *Rx* and *Ry* genes in potato for resistance to *X* and *Y* viruses, respectively, the dominant resistance gene for the Milo disease in *Sorghum* (jowar) and the recessive resistance to Victoria blight in oats are not matched by the pathogen with virulence genes and, therefore, are stable. Some other examples are wilt resistance in watermelon (*Citrullus vulgaris*), resistance to root-knot nematode in Lima beans (*P. lunatus*) and resistance to cabbage yellows in cabbage.

An interesting case of a resistance gene unmatched by the pathogen has been recently reported for leaf rust in wheat. This

Table 21.6. Host-pathogen interaction in case of linseed (*L. usitatissimum*) rust ; an example of gene-for-gene relationship postulated by Flor.

Host Genotype	*Pathogen genotype*	*Disease reaction*
nn pp	A_NA_N A_PA_P	Susceptible
	A_NA_N a_Pa_P	
	a_Na_N A_PA_P	
	a_Na_N a_Pa_P	
NN pp	A_NA_N A_PA_P	Resistant
	A_NA_N a_Pa_P	
	a_Na_N A_PA_P	Susceptible
	a_Na_N a_Pa_P	
nn PP	A_NA_N A_PA_P	Resistant
	a_Na_N A_PA_P	
	A_NA_N a_Pa_P	Susceptible
	a_Na_N a_Pa_P	
NN PP	A_NA_N A_PA_P	Resistant
	A_NA_N a_Pa_P	
	a_Na_N A_PA_P	Susceptible
	a_Na_N a_Pa_P	

Virulence is due to recessive genes designated *a* ; the symbol for the resistance gene toward which it determines virulence is used as a subscript, *e.g.*, a_N denotes the gene that governs virulence toward the resistance gene *N*. Diploid genotypes for the pathogen are written because the uredio-spores that spread the infection are dicaryotic. In heterozygous state, *e.g.*, A_N a_N, the pathogen would be avirulent since virulence is recessive. In case of pathogens whose infection spores are haploid and monocaryotic, haploid genotypes of the pathogen would be used, *e.g.*, a_N a_P, instead of a_N a_N a_P a_P.

resistance allele was induced by a mutagen in Kharchia Local variety of wheat, and is dominant to the allele for susceptibility. It has conferred resistance against all the natural races of leaf rust in India, Australia and Canada, and also against several laboratory synthesized races. Two vital questions remain to be answered in this case. The *first* question concerns the identity of this resistance gene : is it an entirely new gene for which the pathogen does not have the corresponding virulence gene, or is it an extremely strong gene so that the race virulent toward this gene does not survive in nature ? The *second* question concerns the stability of this gene with time ; will it remain stable or will it be overcome by the pathogen in due course of time ?

Molecular Basis for Gene-for-Gene Relationship. The gene-for-gene relationship is due to specific interactions between the products of genes governing host resistance and those conditioning pathogenecity. For example, *Helminthosporium sacchari*, causing leaf blight of sugarcane, produces a toxin which binds to a protein present in the plasma membrane of sugarcane cells ; this interaction leads to the susceptible response of the host. But in many other cases, such an

interaction produces resistant response. Thus on the basis of the molecular interactions involved in producing resistant/susceptible responses in the host, the gene-for-gene relationship may be classified into two general groups : (1) incompatible reaction and (2) compatible reaction.

Table 21.7. The incompatibility (occurring in many biotrophic pathogens) and compatibility (found in many heterotrophic pathogens) types of gene-for-gene relationship in host-pathogen interactions. One gene for resistance (dominant) and one gene for virulence (recessive) are assumed.

Resistance gene	*Virulence gene*			
	Incompatibility		*Compatibility*	
	P_1	p_1	P_1	p_1
R_1	R*	S	R	R
r_1	S	S	R	S

* *R* represents resistant response, while *S* represents susceptible response of the host.

Incompatible Reaction. This type of gene-for-gene relationship is found in biotrophic pathogens (obligate parasites), *e.g.*, rusts, smuts etc. The alleles for resistance in the host (*R*) and those for avirulence in the pathogen (*P*) produce specific compounds which recognise each other ; when these specific compounds interact, they produce resistant response in the host. The alleles for susceptibility (*r*) in the host as well as those for virulence (*p*) in the pathogen either produce these compounds in a modified state or do not produce them at all. Consequently, the products of *p* and *r* are unable to recognise each other, and there is no interaction between them. The lack of interaction between the products of the genes for resistance and pathogenicity produces susceptible reaction in the host. As a result, three of the for combinations between the two alleles of a single gene for resistance (host) and the two alleles of a single gene for pathogenicity (pathogen) would be susceptible (Table 21.7). Only one of these four combinations would lead to the resistant response, since the products of R_1 and P_1 would recognise and interact with each other. Clearly, this system is akin to the self-incompatibility system of higher plants.

Compatible Reaction. In this system, the alleles for susceptibility (*r*) in the host and those for virulence (*p*) in the pathogen produce specific compounds which interact with each other to produce the susceptible response. A lack of interaction between the products of *r* and *p* specifies resistant response of the host. This type of gene-for-gene relationship is found in heterotrophic pathogens (facultative parasites), *e.g.*, *Helminthosporium* leaf blights (sugarcane, maize etc.), Victoria blight of oats etc. The allele for resistance in the host (*R*) produces a modified substance (a protein in the case of sugarcane-*Helminthosporium* system) which is unable to recognise and interact with the product of the allele for virulence in the pathogen. Similarly, the allele for avirulence in the pathogen (*P*) specifies a

modified product, generally a toxin, which is not able to interact with the product of the allele for susceptibility in the host. Thus three of the four combinations between the two alleles of a single gene for resistance and the two alleles of a single gene for pathogenicity would produce resistant response in the host ; only one of the four combinations would lead to susceptibility (Table 21.1).

Vertifolia Effect. Van der Plank introduced the term *Vertifolia effect* derived from the name of a German potato variety Vertifolia having the late blight resistance genes R_3 and R_4. The variety became susceptible when the appropriate pathotype $P_{(3,\ 4)}$ evolved in the pathogen leading to a nearly complete failure of the crop ; such a total failure of vertical resistance leading to a disease epidemic is known as Vertifolia effect.

Thus Vertifolia effect refers to an epidemic development in a variety carrying vertical resistance genes (oligogenes) leading to heavy economic losses. This failure occurs because of two reasons : (1) the level of horizontal resistance in varieties carrying oligogenes is usually low, and (2) the pathogen is able to evolve the virulent pathotype.

Strong and Weak Oligogenes. These terms were introduced by Van der Plank and are often misused. These terms do not refer to the characteristics of resistance genes, but to those of the pathotypes virulent toward these genes. *When the pathotype virulent towards a resistance gene has poor survival value in competition with other pathotypes in the absence of that resistance gene in a host population, the resistance gene is known as a* strong gene. *But when the pathotype specific (virulent) to a resistance gene is a strong competitor in the absence of that gene, the gene is termed as a weak gene.* Thus the pathotype specific to a strong gene is a weak competitor, while that specific to a weak gene is a strong competitor in the absence of that specific resistance gene in the host population. However, when this resistance gene is present, the pathotype specific to it is strongly selected for and the other pathotypes are selected against. Weak resistance genes are of little use in crop improvement, but the strong genes serve a useful purpose. *Sr 6* gene for resistance to stem rust in wheat (*T. aestivum*) is a strong resistance gene since the pathotype virulent toward it is a poor competitor when *Sr 6* is absent in a wheat population.

Polygenic Inheritance

In this case. disease resistance is governed by many genes with small effects, and a continuous variation for disease reaction is produced. The disease reaction is not classifiable into clear-cut resistant and susceptible classes as is the case with oligogenes. The genes show both additive and nonadditive effects, and there is a large environmental effect, as is the case with other quantitative traits. Polygenic resistance is quantitatively inherited, and usually has a large SCA component. The mechanism of resistance is not clearly known ; resistance to infection, slow growth of the pathogen and

slow spore production are generally involved. In this case, there is some spore production, *i.e.*, $r>0$. In some cases, the polygenic inheritance may have a oligogenic component, the oligogenes acting in an additive manner, *e.g.*, bacterial blight resistance in cotton. *Presumably, polygenic resistance does not show pathotype-specificity as against the oligogenic resistance. It may be seen that polygenic resistance is almost the same as horizontal resistance.*

Cytoplasmic Inheritance

In some cases, resistance is determined by cytoplasmic gene(s) or plasma gene(s). For example, maize strains having the T male sterile cytoplasm (cms-T) in maize are extremely susceptible to *Helminthosporium* leaf blight, while those having the normal or non-T cytoplasm are resistant to this disease. Cases of cytoplasmic inheritance of resistance are rare, and the above is the only good example.

SOURCES OF DISEASE RESISTANCE

Resistance to diseases may be obtained from four different sources : (1) a known variety, (2) germplasm collection, (3) related species and (4) mutations.

A Known Variety. Disease reactions of most of the cultivated varieties are documented, and a breeder may find the resistance he needs in a cultivated variety. Resistant plants were isolated from commercial varieties in the cases of cabbage yellows in cabbage (*B. oleracea*), curly-top resistance in sugar beets (*B. vulgaris*), mildew and leaf spot resistance in alfalfa (*M. sativa*) etc. These provided the basis for new resistant varieties of these crops.

Germplasm Collection. When resistance to a new disease or a new pathotype of a disease is not known in a cultivated variety, germplasm collections should be screened. There are numerous instances where resistance to a disease was obtained from germplasm collections, *e.g.*, resistances to net-blotch in barley (*H. vulgare*), resistance to wilt in watermelon etc.

Related Species. Often resistance to a disease may not be present in the varieties of the concerned crop species. In such cases, it would be necessary to transfer resistance genes from related species through interspecific hybridization (Chapter 25). Despite many problems in such gene transfers, it has been successfully and extensively used in many cases. A notable case is the resistance to stem, leaf and stripe rusts of wheat ; a large number of resistance genes, *e.g.*, *Lr 9*, *Lr 19*, *Lr 22a*, *Lr 22b*, *Lr 24*, *Lr 25*, *Lr 26*, *Lr 28* and *Lr 29* for leaf rust resistance, and *Sr 21*, *Sr 24*, *Sr 25*, *Sr 26*, *Sr 27*, *Sr 31*, *Sr 32*, *Sr 34* and *Sr 35* for stem rust resistance have been transferred from related species to wheat, and some of them have been widely used in crop improvement programmes. Resistance to yellow mosaic has been transferred from the wild species *Abelmoschas manihot* ; a yellow mosaic resistant variety of bhindi (*Abelmoschas esculentus*), Parbhani Kranti, has resulted from this programme.

Mutations. Resistance to some diseases may be obtained through mutations arising spontaneously, in some cases, or induced through mutagen treatments, as often is the case. Resistance to Victoria blight (*Helminthosporium victoriae*) in oats (*A. sativa*) was induced by irradiation with X-rays or thermal neutrons ; resistant mutants were also isolated spontaneously in low frequencies. Some other cases of induced mutations for resistance are : resistance to stripe rust in wheat, crown rust in oats, mildew in barley, rust in linseed (*L. usitatissimum*), and leaf spot (tikka) and stem rot in peanuts (*A. hypogaea*).

METHODS OF BREEDING FOR DISEASE RESISTANCE

The methods of breeding for disease resistance are essentially the same as those for other agronomic characters. The following breeding methods have been commonly used : selection, introduction, mutation and hybridization.

Selection. Selection of resistant plants from a commercial variety is the cheapest and the quickest method of developing a resistant variety. The method has been useful in many cases in the past, but it has only a limited usefulness at the present level of crop improvement. Kufri Red potato is a disease resistant selection from Darjeeling Red Round. Other examples include resistance to curly-top in sugar beets, to mildew and leaf spot in alfalfa, to cabbage yellows in cabbage and to *Periconia* root rot in sorghum. Pusa Sawani *bhindi* (*A. esculentus*) is a selection from a collection from Bihar ; it is comparatively resistant to yellow mosaic under field conditions. Cotton (*G. hirsutum*) variety MCU 1 (Madras Combodia Uganda 1) was selected from the variety Coimbatore 4 (CO 4) for resistance to black-arm ; it has an acceptable level of resistance under field conditions.

Introduction. Resistant varieties may be introduced for cultivation in a new area. This offers a relatively simple and quick means of obtaining resistant varieties. Introduction is quick, but has certain limitations. *Firstly*, the introduced varieties may not perform well, *secondly*, they may become susceptible in the new environment, and *thirdly*, they may be susceptible to other diseases common in the new area. For example, Kenya wheats (*T. aestivum*) introduced in India were rust resistant but highly susceptible to loose smut. Similarly, the American cotton (*G. hirsutum*) varieties were susceptible to red blight.

Introductions have served as a useful method of disease control. For example, Ridley wheat introduced from Australia has been useful as a rust resistant variety. Early varieties of groundnut (*A. hypogaea*) introduced from USA have been resistant to leaf spot or *tikka* disease (*Cercospora arachidicola*). Kalyan Sona and Sonalika wheat varieties originated from the segregating material introduced from CIMMYT, Mexico, and were rust resistant. Introductions also serve as sources of resistance in breeding programmes. For

example, African bajra (*P. americanum*) introductions have been used in developing downey mildew resistant male sterile lines (Tift 23A cytoplasm) for use in hybrid bajra production. This has been a crucial development in the hybrid bajra programme since the original male sterile lines Tift 23A and $23D_2A$ were extremely susceptible to downy mildew.

Mutation. We have considered the usefulness of spontaneous as well as induced mutations in the breeding for disease resistance. The methods of mutagenesis will be discussed in Chapter 23.

Hybridization. Hybridization is the most common method of breeding for disease resistance. Hybridization serves two chief purposes : (1) transfer of disease resistance from an agronomically undesirable variety to a susceptible but otherwise desirable variety (by backcross method), and (2) combining disease resistance and some other desirable characters of one variety with the superior characteristics of another variety (by pedigree method). In the first case (backcross method), the new variety is agronomically the same as the susceptible variety, but is disease resistant. In the second case (pedigree method), on the other hand, the new variety is expected to be superior to both the parents in agronomic characteristics and at the same time would be disease resistant.

In both the cases, one parent is selected for disease resistance ; it should have a high intensity of resistance to as many races of the pathogen as possible, and the resistance should be governed by few oligogenes. When the resistant variety is unadapted and agronomically undesirable, backcross method is the obvious choice. But when the resistant variety is well adapted and has some other desirable features as well, the pedigree method of breeding is preferred.

Pedigree method. The pedigree method is quite suited for breeding for horizontal or polygenic resistance as backcross method, in such cases, is of limited value. Pedigree method for breeding of disease resistance is not materially different from the method used for other quantitative traits, *e.g.*, yield. *In breeding for disease resistance, artificial disease epidemics are generally produced to help in the selection for disease resistance (see later).* Therefore, we shall not examine the pedigree method of breeding for disease resistance in any detail. A vast majority of disease resistant commercial varieties have been developed through the pedigree method of breeding, *e. g.*, Kalyan Sona, Sonalika, Malviya 12, Malviya 37 and many other wheat varieties. *G. hirsutum* (American cotton) variety Laxmi, resistant to red leaf blight, was developed through pedigree method from the cross between susceptible parent Gadag 1 and the resistant parent Coimbatore Combodia 2.

Backcross Method. The backcross method is useful in transferring genes for resistance from a variety that is undesirable in agronomic characteristics to a susceptible variety which is widely adapted and is agronomically highly desirable. The backcross programme would

differ depending upon the allelic relationship of the resistance gene, *i.e.*, whether it is recessive or dominant to the allele for susceptibility. The backcross method as applicable to dominant (Fig. 15.2) and to recessive (Fig. 15.3) genes for rust resistance is outlined in chapter 15 ; it will not be discussed any further.

Generally, 5-6 backcrosses are made. A selection for the plant type of the recurrent parent (the susceptible variety) during backcrossing, particularly in BC_2 and BC_3, is effective in making the backross progeny resemble the recurrent parent rather rapidly. At the end of the backcross programme, the progeny are selfed and resistant plants are selected. Progenies derived from different resistant plants that are identical in agronomic characteristics are usually bulked to produce the new disease resistant variety. The new variety would be almost identical to the recurrent parent, except for the disease resistance ; hence extensive yield trials are usually not required before its release for commercial cultivation.

TESTING FOR DISEASE RESISTANCE

Effective breeding for disease resistance depends upon the identification of resistant plants from the susceptible ones with a degree of certainty. The incidence of diseases under natural conditions varies from year to year and their spread is generally uneven in the field. Many susceptible plants may be classified as resistant in such conditions. Therefore, it is often necessary to create *artificial epiphytotics or epidemics*, that is a heavy incidence of a disease by artificial means. The success in producing artificial epidemics depends upon a precise knowledge of the pathogen and of the conditions required for a heavy infection to occur (these conditions must be provided). Further, a knowledge of pathotype differentiation in the pathogen, and of the prevalent pathotypes is also essential for a meaningful breeding programme. Therefore, most resistance breeding programmes are based on cooperative efforts of plant breeders and plant pathologists.

Disease resistance tests may be carried out in the field or in a glass-house. Glass-house tests are more reliable since the favourable environment for disease development can be provided more readily in a glass-house than in a field. An optimum temperature and humidity are essential, though the optimum conditions for one disease may vary considerably from that for another. The general inoculation techniques used for the various catagories of pathogens are briefly outlined below ; the exact details of the techniques are beyond the scope of this text.

Soil-Borne Diseases. Diseases, such as, wilts, root rot, damping off, etc., are produced by fungi present in the soil. Generally, *sick-plots* are created for testing resistance to such diseases. *Sick-plots* are fields that have a sufficient inoculum load of the pathogen to infect all the susceptible plants of the host population. Sick-plots may be produced by mixing the soil from other sick-plots, by adding the

remains of diseased plants, or by adding inoculum produced in a laboratory on host seeds, seedlings or on a nutrient medium. The pathogen inoculum may be increased by growing a susceptible variety for one or more years (the most commonly used technique). Glass-house tests may be carried out using the soil from a sick-plot.

Air-Borne Diseases. A vast majority of the diseases, *e.g.*, rusts, smuts, mildews, blights, leaf spots etc., are produced by air-borne fungi. Inoculation of such a disease may be done by dusting spores from infected plants onto test plants, spraying a suspension of spores or mycelium, injecting a spore suspension into individual plants or leaves, or, in some cases, by planting rows of a highly susceptible variety, *i.e.*, *infector*, to produce a large inoculum which is spread by natural forces, *e.g.*, wind. In the case of wheat rusts, infectors are commonly used ; Agra Local wheat is the common infector for all the three rusts. In the case of air-borne fungi infecting ovary, *e.g.*, loose smut of wheat and barely, spores are introduced in the flowers at the time of anthesis with the help of a forcep or a hypodermic needle.

Seed-Borne Diseases. Some diseases are seed-borne, *e.g.*, some smuts, wheat bunt etc. In such cases, dry spores may be dusted on the seeds before planting, *e.g.*, in the case of sorghum smut. Alternatively, the seeds may be soaked in a suspension of pothogen spores under vacuum, *e.g.*, oat smut, covered smut of barley etc. Vacuum removes the air from seeds and facilitates the entry of spores underneath the husk.

Insect-Transmitted Diseases. Most of the viral diseases are transmitted by insects ; such insects are known as *vectors.* Insect vectors include aphids, leaf hoppers, etc. For inoculation of such diseases, insects feeding on diseased plants are collected and transferred to healthy plants. Alternatively, juice is extracted from the diseased plant tissues and rubbed on healthy plant tissues after mechanical injury has been inflicted to the latter. Mechanical injury is necessary to facilitate the entry of pathogens in the host tissues.

The foregoing is a very general description of the inoculation methods for creating of artificial disease epidemics. The details, however, vary to a great deal from one pathogen to another. For disease resistance breeding, a thorough knowledge of the pathogen is, therefore, a prerequisite.

DISEASE EPIDEMICS

An epidemic is a severe outbreak of disease beginning from a low level of infection. Epidemics are of a common occurrence in the case of air-borne fungi. Soil-borne fungi show epidemics when they are introduced in a new locality, but later on they show chronic levels of infection. An epidemic progresses from a low infection level; often a very low level of infection may initiate an epidemic. For example, an epidemic of potato leaf blight may be initiated by a single

infector plant per square kilometre. The development of an epidemic primarily depends upon the rate of spore production (r); r determines the severity, earliness and the rate of the development of epidemics. The severity of an epidemic at the time of maturity depends not only on r, but also on the time allowed for the epidemic to develop. Thus the severity of an epidemic may be substantially reduced by reducing r. It is not necessary to reduce r to zero ; even if $r = 0.4$, the development of epidemics is considerably delayed. Alternatively, in many cases, the severity of epidemics at the time of maturity may be reduced by breeding early varieties. In practice, the breeder often uses a combination of both these approaches.

Causes of Epidemics

Natural populations suffer from diseases, but they rarely have epidemics: Epidemics are the result of modern agricultural technology. The factors that promote disease epidemics are : narrow genetic base of the cultivated crops, large acreages under single varieties, introduced pathogens, failure of vertical resistance genes and inadvertent breeding for susceptibility.

Narrow Genetic Base. The commercial cultivars are generally purelines, clones or hybrid varieties and consequently are more or less genetically uniform. The population is, therefore, not flexible enough genetically to adapt itself to the pathogen, or, so to say, coevolve with the pathogen.

Large Acreages under Single Varieties. The host crops are generally present in contiguous fields in large areas, and often these are planted to a single variety. This facilitates the spread of pathogens once infection is initiated.

Introduced Pathogens. When a new pathogen or a new pathotype of an existing pathogen is introduced from outside, the local crop varieties may not be resistant to it. Usually, an epidemic would result until the host has evolved resistance to the new pathogen, *e.g.*, maize in West Africa in relation to rust, potato late blight in Europe and many other examples.

Failure of Vertical Resistance. The breeding for vertical resistance generally makes a selection for horizontal resistance impossible. Thus varieties having vertical resistance genes usually have low levels of horizontal resistance. In such cases, when the vertical resistance fails, an epidemic is the result. The Vertifolia potato variety furnishes a striking example. The failure of vertical resistance is a common feature leading to epidemic cycles. The average effectiveness of a major gene for rust resistance in wheat is supposed to last for 5 years, but for many genes this period may be much shorter than this.

Inadvertant Breeding for Susceptibility. In several cases, a variety may be produced that is susceptible to a thus far minor disease.

This minor disease then produces an epidemic due to a widespead cultivation of susceptible host genotypes. Victoria oat (*A. sativa*), bred for crown-rust resistance, had the dominant gene for susceptibility to *Helminthosporium* blight, an unknown disease upto that time. The male sterile T-cytoplasm in maize (*Z. mays*) was susceptible to *Helminthosporium* leaf blight. The Tift 23A male sterile inbred used as the female parent of the hybrid bajra (*P. americanum*) varieties in India was extremely susceptible to downy mildew and ergot. In all the three cases cited above, minor diseases became of severe epidemic proportions due to the high susceptibility of prevalent host varieties.

Prevention of Epidemics

The prevention of epidemics has been of great concern, and several possible measures have been suggested for this purpose. These measures are, genetic diversity within varieties, between varieties and in the breeding reserves, and the use of horizontal resistance.

Genetic Diversity Employing Vertical Resistance. Strong genes for vertical resistance may be employed in different ways to check epidemics. *Firstly*, they may be used in *alternate years* so that the virulent pathotype against any one of them does not evolve and does not survive even if it did evolve. *Secondly*, they may be used for planting in different geographical regions ; these regions would run perpendicular to the direction of movement of the pathogen. Thus each strong gene would act as a sieve for the pathotype virulent on the succeeding one(s). A good example is provided by the wheat stem rust resistance gene *Sr6* in North America. There the rust migrates northward, and before reaching spring wheats carrying the gene *Sr6*, it passes through the winter wheat area lacking *Sr6*. Since the pathotype virulent toward *Sr 6*, is a poor competitor, the winter wheats act as a sieve for it. *Sr6*, as a result, has been the most effective gene against stem rust in North America. However, these two measures depend on a special condition that generally cannot be ensured, that is, planting of specific varieties in specified temporal or spatial order. Therefore, they cannot be relied upon.

The *third* measure involves the planting of different closely situated fields to different varieties carrying different resistance genes. This should reduce the severity of an epidemic, but has not been practically tried. The *fourth* measure is the use of multiline varieties, each pureline of the multiline carrying a different resistance gene (Chapter 16). Multiline varieties may be expected to reduce the severity of an epidemic since only one or a few of the component lines may be expected to become susceptible at any one time. Multilines have not been widely used commercially. Three Indian wheat multiline varieties are KSML3, KML 7406 and MIKS11 ; both are based on the popular wheat variety Kalyan Sona. However, these multilines have not become popular among the farmers.

Breeding for Horizontal Resistance. The advantages sought by the various measures outlined above using vertical resistance are all

provided for by horizontal resistance. In certain crop species, *e.g.*, in case of potato for late blight, horizontal resistance is the sole objective of breeders. There is a growing awareness for the value of horizontal resistance and indications are that it will find greater applications in more and more crop species in the future. Some people believe that the ultimate answer to resistance breeding lies in a wise use of horizontal resistance. Simmonds (1978) states that 'there is a substantial truth in the idea that we have the diseases to which we breed susceptibility'.

SUMMARY

Disease is an abnormal condition produced by an organism, *e.g.*, fungi-bacteria, viruses, nematodes and insects. Diseases reduce yield by damaging various plant parts or by reducing general vigour. There are generally several pathotypes within a pathogen species. A *pathotype* is a strain of pathogen virulent toward a specific resistance gene in the host (similar in meaning to *physiological race*, but not the same). The pathogen produces new pathotypes by sexual reproduction, heterocaryosis, parasexual reproduction and mutation. *Pathogenicity* (ability of the pathogen to infect the host and produce disease) is genetically controlled ; pathogenicity is generally recessive to nonpathogenicity. *Resistance* of the host is the ability of the host to prevent the pathogen from producing disease. Disease resistance is generally dominant to susceptibility, but in some cases resistance is recessive. The host-pathogen relationship is described by the *gene-for gene* hypothesis of Flor : *for every resistance gene present in the host, the pathogen has a gene for virulence* (*ability to produce disease*). A susceptible reaction would result only if the resistance gene is matched by the specific virulence gene in the pathogen. The hypothesis is widely accepted, but there are cases in which the pathogen is unable to match the resistance gene present in the host.

A disease develops through four stages: contact, infection, establishment and development. Susceptible hosts may be able to *escape disease* due to unfavourable environment for infection, earliness, change in the date of planting, change in the site of planting, control of insect vectors and chemical disease control. Disease resistance may be immune reaction (no disease, $r=0$), resistance (less disease, $r>0$ but less than 1) or tolerance (high disease incidence, but small loss in yield). Resistance is also classified as vertical and horizontal *Vertical resistance* is oligogenic, pathotype-specific and generally shows immunity. Horizontal resistance is polygenic, pathotype non-specific and acts by reducing r, *i.e.*, $r>0$ but <1. The mechanism of disease resistance may be mechanical, due to hypersensitivity, antibiosis or nutritional in nature.

Genetically, disease resistance may show oligogenic, polygenic or cytoplasmic inheritance. Few oligogenes are strong, while many are weak (terms refer to the competitive abilities of the pathotypes virulent against the resistance genes). Oligogenes often show *Vertifolia effect* : severe epidemic development when the pathotype virulent toward the resistance gene is evolved. For breeding, disease resistance may be obtained from a known variety, from germplasm collections, from related species or through mutation. The methods of breeding for disease resistance include selection from a heterogeneous source population, introduction of a resistant variety, mutation and hybridization. Progeny from hybridization may be handled through backcross of pedigree method. An important component of breeding for disease resistance is testing for resistance by creating artificial epiphytotics : *Epiphytotics* are created in different ways for different diseases depending upon the nature of the pathogen and the environment necessary for the disease development.

Generally, diseases develop from a low infection level to a severe level ; it is known as *epidemic*. An epidemic may start from a very low infection level, *e.g.*, 1 in 10^7 plants. An epidemic results due to a narrow genetic base of cultivated varieties, large areas planted to a single variety, introduction of a new pathogen, and unknowingly breeding for susceptibility. Suggested measures for preventing epidemics are based on vertical resistance or horizontal resistance. Few strong oligogenes may be used for planting in alternate years in different geographical areas, in different fields or in a mixture, *e.g.*, a multiline variety. These measures are expected to imitate horizontal resistance, which appears to be the answer for preventing epidemics, and signs are that in future it would become increasingly important in breeding programmes.

QUESTIONS

1. Differentiate between : (i) horizontal and vertical resistance, (ii) pathotypes and physiological races. (iii) disease resistance and disease escape, (iv) virulence and avirulence, (v) immunity and resistance, (vi) susceptibility and tolerance, (vii) resistance and pathogenicity, and (vii) strong and weak resistance genes.
2. Briefly describe the mechanisms by which pathogens produce new pathotypes.
3. The genetics of disease resistance is limited by the availability of new genes for virulence, while that of pathogenicity is limited by new genes for resistance. Comment with the help of suitable examples.
4. Define the following : (i) differential hosts, (ii) antibiosis, (iii) hypersensitive reaction, (iv) vertifolia effect, (v) epidemics, and (vi) sick-plots.
5. Write short notes on the following : (i) gene-for-gene relationship, (ii) physiological races, (iii) Ideal differential hosts, (iv) artificial epiphytotics (or epidemics), and (v) epidemics.
6. List the sources of disease resistance. Discuss any two of them in detail with the help of suitable examples.
7. List the methods of breeding for disease resistance. Describe the back-cross method of breeding if the disease resistance is governed by a recessive (or dominant) gene.
8. Effective breeding for disease resistance depends on a uniform spread of the disease in the breeding material. Discuss the above statement.
9. Describe the various methods for creating artificial epiphytotics.
10. Briefly describe the various mechanisms of disease resistance in plants.
11. Define epidemic. Discuss the causes of epidemics and the possible methods for their control.

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CHAPTER 22

Breeding for Insect Resistance

All the crop species are attacked by insects, but the degree of damage to as well as the number of insect species attacking different crop species vary considerably. For example, cotton is attacked by more than 160 species of insects ; of these about a dozen are major insect pests. An effective pest control measure is the basic requirement for a good cotton crop ; this is clear from the fact that 60% of all the pesticides used in the world is applied to cotton. On the other hand, crops like wheat and barley are attacked by a few insect pests and none of these cause a serious damage. It may be noted that many insect pests have a rather narrow host range, *i.e.*, they attack only a limited number of plant species. But many other insects feed on several crop species, *e.g.*, red cotton bug, *Heliothis* etc. Furthermore, an insect may move on to a new host species if its normal host crop is not available.

Once it was hoped that chemical control measures will effectively control, or even eliminate the insect pests. But the experience with pesticides has shown that such a hope was entirely misplaced. Extensive pesticide application (1) increases the cost of production of crops, (2) reduces the population of natural enemies (predators and parasites) of insect pests, (3) leads to the development of pesticide-resistant races of insects, and (4) pollutes the environment (the most tragic and horrifying example is the Bhopal Gas Tragedy of December, 1984). Most entomologists now speak of '*pest management*' in the place of '*pest control*'. Pest management involves, in addition to pesticide application, several divergent measures to minimise the losses due to insect pests. *Insect resistant crop varieties, where available, form an important component of pest management schedules. Insect resistance is the property of a variety of a host crop due to which it is attacked by an insect pest to a significantly lower degree than other varieties of the same host. From a practical view point, an insect resistant variety produces relatively larger yields of good quality than susceptible varieties with the same level of initial infestation (insect attack) under comparable environmental conditions.* Thus, resistance is a relative property and can be defined only in comparison to other more susceptible varieties (Maxwell *et al.*, 1972).

Losses Due to Insects

Insect pests may be grouped into two classes on the basis of their mode of feeding : (1) *sucking pests* suck the cell sap, *e.g.*, aphids, jassids, thrips, white fly, mites etc., and (2) *tissue feeders* feed on the various plant parts, *e.g.*, stem or shoot borer, root borer, fruit borer, leaf hoppers, weevils, beetles etc. Both the types of insects reduce the quality as well as the quantity of crop yields. The loss in quality of the produce is more frequent and often of a greater concern than that of quantity *per se*. Insect infestation leads to one or more of the following *direct damages* : (1) reduced plant growth or stunting, (2) damage to leaf, stem, branch, flower buds, flower, vegetative buds, fruits and seeds, (3) premature defoliation of leaves, and even (4) wilting of plants. The degree of damage depends primarily on the intensity of attack and the susceptibility of the host strain. In addition, insects also cause indirect damage. Many insects, *e.g.*, aphids, mites, white fly etc., transmit plant viruses, *i.e.*, serve as vectors of pathogens. Further, injuries caused by insects make the plants more vulnerable to attacks by fungal and bacterial pathogens.

The estimated global average loss due to insect pests in the potential yields of all the crops is 14 per cent (Cramer, 1967). The estimated losses in individual crops vary from 5 per cent in wheat to 26.7 per cent in rice, and still more in crops like cotton and sugarcane. In spite of the widespread use of insecticides and other control measures, the global average loss in most of the major crops exceeds 5 per cent. In India, losses due to insect pests range from 10 to 20 per cent. Losses due to insect pests are much more in tropical and subtropical than in temperate regions since high temperature and humidity promote insect activity and reproduction. In cases of severe insect attacks, the yield losses may be upto 90 per cent. There are several cases where a crop species was virtually eliminated due to an insect pest. An excellent example is furnished by grape in France ; about 120 years ago its cultivation was virtually wiped out by *Phylloxera vertifoliae*. The grape crop in France was saved by the use of *P. vertifoliae*-resistant root-stocks introduced from U.S.A.

Genetic Variability in Insect Pests

Strains of many insect pests, *e.g.*, aphids, leaf and plant hoppers of rice etc., differ in their ability to infest or attack varieties of the same host species that differ in their resistance to that pest. Such strains of an insect pest are referred to as *biotypes* ; the situation is similar to the physiological differentiation in plant pathogens (Chapter 21). However, biotype differentiation in insect pests appears to be much less frequent than physiological differentiation in the case of pathogens, and its consequences are generally not as striking. The reasons for this are not very clear, but it may be due to one or more of the following. *Firstly*, the number of offspring produced in each generation by an insect pest is generally only a

small fraction of the astronomical number produced by plant pathogens, more particularly fungal pathogens. In addition, plant pathogens produce few to several hundred generations (bacteria and viruses) in a single crop season as compared to one to few for most insect pests. As a result, the chances of origin of genetic variation through mutation and other mechanisms is much smaller in the case of insect pests than in plant pathogens. *Secondly*, insect resistance generally involves some morphological or some nonspecialized biochemical/physiological factors of the host plant. Overcoming of such mechanisms of resistance would require a change in the habit or the physiology of the insect pest, which may be expected to be governed by more than one gene. As a result, biotype differentiation of insect pests may be expected to be much less frequent than physiological differentiation of plant pathogens since the latter generally involves a single gene. *Thirdly*, biotype differentiation in insect pests can be studied only when varieties of the host differing for resistance genes are available. Relatively fewer cases of resistance are known for insect pests than those for plant pathogens. *Fourthly*, insect resistant varieties have generally not been cultivated as widely as disease resistant ones. Therefore, the selection pressure for the development of new biotypes of insect pests has not been as great as that for new pathotypes in the case of plant pathogens. But it may be pointed out that insect resistant varieties of many crop species have been extensively grown for several years, but insect biotypes capable of attacking them have not emerged so far. Similar cases are also known for several plant diseases as well. *Finally*, insect resistance has not been as widely investigated as disease resistance as a consequence of the relatively smaller economic losses caused by insects. This itself may be a sound reason for our limited knowledge on the subject. More cases of bio-type differentiation in insects are becoming known as they are being intensively investigated.

An insect resistant variety may be attacked by a true resistance-breaking biotype or merely by a more vigorous biotype. A *true resistance-breaking biotype* is adapted to overcome a specific resistance mechanism of the host plant. As a result, it is more successful in attacking varieties which possess that specific resistance mechanism than other host varieties. This gives rise to host-insect pest interaction akin to host-pathogen interaction, *e.g.*, biotypes of aphids and varieties of raspberry, biotypes of leaf and plant hoppers and varieties of rice etc. Sometimes, the biotype attacking a resistant variety may not be a resistance-breaking biotype, but it may simply be a *highly vigorous biotype.* Such a biotype attacks both resistant and susceptible varieties of the host more successfully than the preexisting biotype(s). Such a biotype lacks any specific adaptation to overcome the resistance mechanism of the resistant host variety and it does not show host-insect pest interaction, *e.g.*, cabbage aphid attacking rape varieties. In addition, a pest may be differentiated into taxonomic races or it may show morphological

variations which may or may not be associated with biotype differentiation. Such variations are known in many insect pests, *e.g.*, wheat Hessian fly. Similarly, a host variety resistant to one insect may be attacked by another insect pest for which it was never resistant. For example, some populations of potato cyst nematode have been found to attack eelworm resistant potato varieties. These considerations should be kept in mind while assessing the biotype differentiation in an insect pest and the resistance of host varieties to various insects.

Mechanism of Insect Resistance

Insect resistance is grouped into four categories : (1) nonpreference, (2) antibiosis, (3) tolerance, and (4) avoidance. The first three types of resistance were recognized by Painter (1951), while the fourth, avoidance, was included subsequently. A given case of insect resistance may exhibit one, more often, two or more of these mechanisms. In some cases of resistance, nonpreference, antibiosis and tolerance are simultaneously involved, *e.g.*, resistance of Brussel's sprouts to cabbage aphid. Such combinations of resistance mechanisms are highly effective and are not easily overcome by the concerned insect pest.

Nonpreference. *Host Varieties exhibiting this type of resistance are unattractive or unsuitable for colonization, oviposition or both by an insect pest.* This type of resistance is also termed as nonacceptance and antixenosis. *Nonacceptance* appears to be more appropriate since in most known examples of this type of resistance the insects will not accept a resistant host variety even if there is no alternative source of food. There is a considerable variation in the degree of nonpreference by insect pests. In some cases, the expression is so strong that the insects walk-off the resistant plants, *e.g* , aphid resistance in raspberry. In some cases, on the other hand, the insects do not walk-off the resistant plants, but they feed for a comparatively shorter period and are generally restless on the resistant plants as compared to on susceptible ones, *e.g.*, aphid resistance in sugarbeet (Russel, 1978). Nonpreference involves various morphological and biochemical features of host plants.

Antibiosis. *Antibiosis refers to an adverse effect of feeding on a resistant host plant on the development and or reproduction of the insect pest. In severe cases, it may even lead to the death of the insect pest.* Antibiosis may involve morphological, physiological or biochemical features of the host plant ; some cases involve a combination of all the three features.

Tolerance. Insect tolerance is similar to disease tolerance (Chapter 21). *An insect tolerant variety is attacked by the insect pest to the same degree as a susceptible variety. But at the same level of infestation, a tolerant variety produces a larger yield than a susceptible variety.* In some cases, tolerant varieties show greater recovery

than susceptible varieties from pest damage, *e.g.*, in the case of shoot borer in sorghum. In other cases, tolerance may be due to the ability of host to suffer less damage by the pest, *e.g.*, aphids in sugarbeet and brassica, and greenbugs in cereals.

Avoidance. Pest avoidance is the same as disease escape and, as such, *it is not a case of true insect resistance.* However, it is often as, or even more, effective as true resistance in protecting a crop from pest damage. Most cases of pest avoidance result from the host plants being at a much less susceptible developmental stage when the pest population is at its peak. For example, early maturing cotton varieties escape pink bollworm infestation which occurs late in the season. In some cases, the crop may be grown in a season when the pest population is very low, *e.g.*, seed-plot technique in potato to avoid aphid infestation.

Nature of Insect Resistance

Insect resistance may involve morphological physiological or biochemical features of the host plant (Table 22.1).

Morphological Factors. A number of morphological factors, *e.g.*, hairiness, colour, thickness and toughness of tissues etc., are known to confer insect resistance.

1. **Hairiness.** Hairiness of leaves is associated with resistance to many insect pests, *e.g.*, in cereals to cereal leaf beetle, in cotton to jassids and in turnip to turnip aphid.

2. **Colour of Plant.** Plant colour may contribute to nonpreference in some cases. For example, red cabbage and red-leaved Brussel's sprouts are less favoured than green varieties by butterflies and certain other lepidoptera for oviposition. Similarly, bollworms prefer green cotton plants to red ones.

3. **Thickness and Toughness of Plant Tissues.** Thick and tough plant tissues present mechanical obstruction to feeding and oviposition and thereby lead to nonpreference as well as antibiosis. For example, thick leaf lamina in cotton contributes to jassid resistance, while solid stem leads to a resistance to wheat stem sawfly. Similarly, thick and tough rind of cotton boll makes it difficult for the bollworm larvae to bore holes and enter the bolls.

4. **Other Characters.** Several other plant traits may contribute to insect resistance. For example, *Gossypium arboreum* varieties with narrow-lobed and leathery leaves are more resistant to jassids than those with broad-lobed and succulent leaves. Cotton varieties with longer pedicels are more resistant to bollworms than those with shorter ones ; a longer pedicel makes the movement of larvae from one boll to another more difficult. Leaves of nectariless cotton varieties are devoid of nector glands ; as a result, they are visited by insects less frequently than the normal plants.

Table 21.1. Nature of resistance to insect pests in some crop plants.

Host crop	*Insect pest*	*Cause of resistance*	*Mechanism of resistance*
Wheat	Hessian fly	H_1 to H_8 genes	Antibiosis
	Stem sawfly	Solid stem	Nonpreference and antibiosis
	Greenbugs	High content of benzyl alcohol	Antibiosis
Barley	Cereal leaf beetle	Hairiness and waxiness of leaves	Tolerance and antibiosis
Rice	Rice stem borer	a. High silica content	Antibiosis
		b. Lignified stem	Nonpreference
		c. More schlerenchymatous tissues in stem	Tolerance
	Brown plant hopper	a. Low asparagine content of leaves	Nonpreference
		b. Red pericarp and purple sigma	Nonpreference
Maize	European corn borer	DIMBOA content in leaves	Antibiosis
	Maize stem borer	High aspartic acid, low nitrogen and sugar contents	Antibiosis
	Corn ear worm	Toughness of husk	Nonpreference
	Corn leaf aphid	High DIMBOA in leaves	Nonpreference and antibiosis
Cotton	Bollworms	a. High gossypol content	Antibiosis
		b. Smooth leaves	Nonpreference
		c. Nectariless	Nonpreference
		d. Earlines	Escape
	Boll weevil	a. Frego bract	Nonpreference
		b. Red plant body	Nonpreference
		c. Earliness	Escape
	Jassids	Hairiness of leaves	Nonpreference
Sugarbeet	Aphids	Low free sugar	Nonpreferense and antibiosis
Alfalfa	Spotted alfalfa aphid and pea aphid	High saponin content	Antibiosis
Brassica	Cabbage aphid	Low sinigrin content	Nonpreference and antibiosis
Potato	Aphid	Sticky trichome exudate	Antibiosis

Physiological Factors. Some physiological factors, *e.g.*, osmotic concentration of cell sap, various exudates etc., may be associated with insect resistance. Leaf hairs of some *Solanum* species secrete gummy exudates. Aphids and colorado beetles get trapped in these exudates and are unable to feed and reproduce. The exudates from secondary trichomes of *Medicago disciformis* leaves have antibiotic effects on alfalfa weevil ; at lower concentrations it retards the growth of weevil, while at higher concentrations it is lethal. In cotton, jassid resistance is associated with high osmotic concentration of the cell sap.

Biochemical Factors. Several biochemical factors are known to be associated with insect resistance in many crops. It is believed that biochemical factors are more important than morphological and physiological factors in conferring nonpreference and antibiosis. A well known example is the association between high concentration of gossypol, a phenolic compound, and resistance to several insect pests in cotton. In rice, high silica content in shoots confers resistance to shoot borer as it causes rapid wearing off of mandibular mouth parts of the pest. Maize varieties resistant to European corn borer show high concentration of DIMBOA (2, 4-dihydroxy, 7-methyl 2H-1, 4-benzoxoxazine, 3 (4H)-one), which is highly distasteful to this pest. Similarly, resistance to greenbugs in wheat and barley is associated with high concentration of benzyl alcohol. High saponin content of leaves and stem of alfalfa is associated with resistance to spotted alfalfa aphid and pea aphid. *Lycopersicon esculentum* has an ethanol soluble compound which is highly toxic to tomato fruit worm and tobacco flea beetle.

Some biochemicals act as feeding stimuli for insects ; a deficiency of such compounds in the host may lead to insect resistance (nonpreference). Asparagine acts as a feeding stimulus to brown plant hopper (BPH) in rice. Varieties having relatively lower concentrations of asparagine are more resistant to BPH than those with higher concentrations. Similarly, simigrin acts as a stimulant to cabbage aphid in brassicas, and a low concentration of this biochemical in the leaves leads to a resistance to this pest.

GENETICS OF INSECT RESISTANCE

Genetics of insect resistance is similar to that of disease resistance. Insect resistance may be governed by (1) oligogenes with large individual effects, (2) polygenes with small additive effects or (3) cytoplasmic genes (Table 22.2).

Oligogenic Resistance. In such cases, insect resistance is governed by one or few major or oligogenes, each gene having a large and identifiable individual effect of resistance. In such cases, the resistance may be conditioned by the dominant or the recessive allele of a gene. The differences between resistant and susceptible plants are generally large and clear-cut. In several cases, resistance is governed by a single gene (*monogenic inheritance*), *e.g.*, in wheat to green bugs, in raspberry to aphids, in rice to plant and leaf hoppers, in cotton to jassids, and in apple to woolly aphis.

Monogenic resistance is simply inherited (3 : 1 ratio), is easily transferred, involves a single feature of the host plant, is often overcome by resistance-breaking biotypes, *i.e.*, is less durable. But some cases of monogenic resistance, *e.g.*, jassid resistance in cotton, are quite durable as they have not been overcome by the pests even after an extensive cultivation of the resistant varieties for substantially long periods. Durable monogenic resistance may be due to one

Table 22.2. Genetics of Insect resistance in some crop plants.

Mode of Genetic control	*Crop species*	*Pest*	*Expression of resistance*	*Biotypes known*
Oligogenic	Wheat	Greenbugs	1d+m	+
	Rice	Brown plant hopper	2D+2d	+
		Gall midge	2D+1d	+
		White-backed plant hopper	3D+1d	+
		Green leaf hopper	6D+1d	+
	Cotton	Jassids	1D+m	−
	Raspberry	Aphids	1D	+
	Apple	Woolly aphis	1D	+
	Wheat	Hessian fly	5D+5d	+
		Stem sawfly	1D+1d	−
	Barley	Greenbugs	2D	+
	Maize	Corn borer	NK	−
	Alfalfa	Pea aphid	1D+1d	−
Polygenic	Wheat	Leaf beetle	NK	−
	Rice	Stem borer	NK	−
	Maize	Ear worm	NK	−
		Leaf aphid	NK	−
	Alfalfa	Spotted aphid	NK	−
	Brassica	Aphid	NK	+
Cytoplasmic	Maize	European corn borer	Cytoplasmic	−
	Lettuce	Root aphid	Cytoplasmic	−

D=dominant, d=recessive, m=modifier, NK=Not known, +=Resistance-breaking biotypes known, −=Resistance-breaking biotypes not known.

or more of the following (1) cultivation of resistant varieties over a relatively small area or time or both, (2) a lack of biotype differentiation in pest species, and (3) the involvement of morphological features of the host in resistance. Adaptation of the pest to a morphological feature may necessitate a change in its habit which may be expected to have a complex genetic control. However, it may be pointed out that similar cases of durable resistance are known for plant diseases as well. Modifying genes are known to affect some genes governing insect resistance, *e.g.*, green bug resistance in wheat, jassid resistance in cotton.

Where resistance is governed by two or more oligogenes, the genes may control a single character of the host plant or each oligogene may govern a different trait. The latter situation is more common, and evolution of resistance breaking biotypes in such cases is much less frequent than that in the cases governed by a single major gene. Examples of such resistance are : in wheat to Hessian and stem sawfly, in barley to green bug, in alfalfa to pea aphid etc. An example of a highly durable resistance of this type is that associated with solid stem in wheat to stem sawfly.

Polygenic Resistance. Polygenic insect resistance is governed by several genes each with a small and usually cumulative effect. Such cases of resistance (1) involve more than one feature of the host

plant, (2) are much more durable than the cases of oligogenic resistance, (3) the differences between resistant and susceptible plants are not clear-cut due to a continuous variation for the trait, and (4) the transfer of resistance is relatively much more difficult than that of oligonenic resistance. Examples of polygenic resistance are : in wheat to cereal leaf beetle, in alfalfa to spotted aphid, in rice to stem borer, in maize to ear worm and leaf aphid etc. Evolution of resistance-breaking biotypes is almost rare as the pest has to adopt to more than one feature of the host plant.

Cytoplasmic Resistance. There are atleast two known cases of insect resistance that are governed by plasmagenes. These cases are, resistance to European corn borer in maize and resistance to root aphid in lettuce. It is likely that more cases of cytoplasmic resistance will be discovered if extensive investigations were made.

SOURCES OF RESISTANCE

Resistance to a given insect pest may be found in (1) a cultivated variety, (2) germplasm of the crop species, or (3) a related wild species.

Cultivated Variety. Resistance to many insect pests may be found in a cultivated variety of the concerned crop. For example, varieties SRT 1, Khandwa 2, DNJ 286 and B 1007 of *G. hirsutum* are good sources of resistance to jassids. Utilization of resistance from such a variety in a crop improvement programme presents few problems as it is present in an agronomically acceptable background. Reactions of cultivated varieties of most of the crops to various insect pests is generally known. Therefore, an extensive search for the required resistance must be made in cultivated varieties of the concerned crop. It is worthwhile to introduce such varieties if they are available in another country.

Germplasm Collection. In case the desired resistance is not available in a cultivated variety, it should be searched in the national and/or world collections of the concerned crop species. There are several examples where the desired resistance was obtained from germplasm collections. Screening of 2000 entries of apple germplasm led to the identification of 14 entries showing very high levels of resistance to the rosy apple aphid, three entries entries immune to green apple aphid and several lines resistant to apple sucker and apple sawfly. Similarly, several cotton strains resistant to jassids have been identified by screening the germplasm. Utilization of insect resistance from germplasm collections in breeding programmes may be more difficult than that from cultivated varieties since the resistant lines may not possess good agronomic characteristics. However, it can be utilized far more easily than the resistance present in a related wild species.

Related Wild Species. In many cases, the desired resistance may not be available in the cultivated crop species. In such cases, the resistance should be searched in the wild relatives of the concerned crop

There are many cases where insect resistance has been transferred from a related wild species to a crop species. For example, resistance to both the species of potato nematodes has been transferred from *Solanum vernei* to potato, resistance to root knot nematode from a related wild species to tobacco, resistance for *Rubus* aphid is found in a related wild species of raspberries, and jassid resistances is known in the wild relatives of cotton, *e.g*, *G. tomentosum*, *G. anomalum* and *G. armourianum*.

BREEDING METHODS FOR INSECT RESISTANCE

The breeding methods for insect resistance are the same as those for disease resistance. One of the following methods may be used for this purpose : (1) introduction, (2) selection, and (3) hybridization. The choice of breeding method in a given case depends on the source of resistance, the mode of its inheritance and the natural mode of pollination of the crop species.

Introduction. An introduced variety resistant to the concerned insect may be released for cultivation if it performs well in the new environment and is agronomically desirable. Thus, it is the quickest and, perhaps, the easiest method of developing an insect resistant variety. But often the introduced variety may not perform well in the new environment and it may be susceptible to the biotypes of the concerned pest prevalent in the area or to new insect pests and/or diseases of the area. There are many examples of introduction of insect resistant varieties. The most striking example is the introduction of *Phylloxera vertifoliae* resistant grape root-stocks from U.S.A. into France ; this saved the grape industry of France from virtual extinction.

Selection. Insect resistant varieties may be present in an existing variety of a crop. In such a case, selection for insect resistance is practised to isolate an insect resistant variety. In self-pollinated crops, mass selection or, more generally, pureline selection is practised for the purpose. Mass selection or, often, recurrent selection is practised for developing an insect resistant variety in cross-pollinated crops. Selection for insect resistance is generally more profitable in cross-pollinated than in self-pollinated crops due to the existence of large genetic variability in the former. An example of selection for insect resistance is that for resistance to potato leaf hopper and spotted alfalfa aphid in two broad-based germplasms of alfalfa ; selection for resistance to both the pests was highly effective in both these populations.

Hybridization. When the desired insect resistance is present in an agronomically inferior variety of the crop or in a related wild species, hybridization is the only course of action for the breeder. Such resistant species/strains are crossed with an agronomically superior and adapted but insect pest susceptible variety of the crop to develop a superior, well-adapted and insect resistant variety. In case the resistant variety is expected to contribute to some of the agronomic

characteristics also of the new variety, the F_2 and the subsequent generations obtained from the cross are handled according to the *pedigree method*. This method is suitable for the resistance governed by either oligogenes or polygenes.. If the resistance is governed by oligogenes, selection for resistance is effectively practised in the F_2 generation. But in the case of polygenic insect resistance, (1) selection for resistance is delayed till the F_3 generation, and (2) a relatively larger number of apparently resistant plants is selected. This is necessary because the differences between resistant and susceptible plants are not clear-cut, and there is a considerable effect of the environment on the expression of resistance.

In many cases, the insect resistant variety may be agronomically undesirable, or the gene for resistance may be present in a related wild species. In such cases, *backcross* is the most suitable breeding method. The resistant variety or the related species is used as the nonrecurrent parent, while an agronomically superior, or high yielding and well-adapted, but susceptible, variety is used as the recurrent parent. The backcross method is commonly used when the resistance is controlled by one or few major genes. But it may be equally useful in cases of polygenic insect resistance as well provided the heritability of the character is high. In general, 5-6 backcrosses are necessary to recover the genotype of the recurrent parent. But in the cases of interspecific transfer of resistance genes, a much larger number of backcrosses may be needed. This is particularly so when it is necessary to break the linkage between the resistance gene and some undesirable genes. Interspecific gene transfers generally suffer from a number of disadvantages discussed in some detail in a later chapter (Chapter 25).

SCREENING TECHNIQUES

The most crucial and, perhaps, the most difficult task in breeding for insect resistance is the identification of insect resistant plants/lines during segregating generations. This requires an equal level of initial infestation of all the plants in the population to ensure that the plants scored as resistant are not escapes. It is often a tedius and time consuming task to ensure an equal initial infestation of all the plants, and in large populations it may not be practically possible. Suitable techniques for screening for resistance to several insects have been developed and successfully used in breeding of insect resistant varieties. The screening for insect resistance may be done either (1) in the field or (2) in a glasshouse.

Field Screening. Screening for insect resistance, particularly in breeding programmes, is usually done in the field since a much larger number of plants can be screened in the field than in a glasshouse. In addition, under field conditions the plants are exposed to other insect pests prevalent in the area ; this reduces the possibility of unintended selection for susceptibility to them. However, field tests are carried out under uncontrolled conditions, and in many cases it may

not be possible to ensure uniform initial infestation of all the plants in a population. As a result, experimental error of field tests is much larger than that of laboratory tests.

There are many techniques designed to promote uniform infestation by an insect pest in the field. (1) The simplest technique consists of interplanting a row of a known susceptible variety between two rows of the plant material being tested. This technique is suitable for air-dispersed insect pests only. (2) Each insect pest occurs much more frequently in some areas of the country. Screening for resistance to the concerned insect in such prone areas would ensure a heavy natural infestation of the test plants. (3) In the case of soil pests, the plant material being tested is planted in the soil known to contain large populations of the concerned pest. Large populations of soil pest can be maintained by regular planting of susceptible varieties in the concerned field. This technique has been used for screening oats and clovers for resistance to eelworm (4) Heavy natural infestations of some pests may occur in a particular season. For example, a much heavier infestation of rice stem borer occurs in the off-season than in the main season crop since in the off-season few alternate hosts are available to the female moths for laying their eggs. Therefore, screening of rice for resistance to stem borer is greatly facilitated in the off-season as compared to that in the main season. (5) Finally, equal number of eggs or larvae may be transferred by hand to each test plant. This ensures uniform initial infestation, and is highly desirable wherever practically feasible. For example, rice plants are infested with stem borer by transferring egg masses, containing about 60 eggs, to each test plant. The number of larvae that hatch are counted and the resistant plants are identified for further testing.

Glasshouse screening. A much smaller number of plants can be screened in a glasshouse, if and where they exist, than in the field. But the results from glasshouse tests are much more reliable than those from field tests since both the environment and the initial level of infestation are more or less uniform for all the plants being tested. Resistance to several pests has been screened in glasshouses. For example, the larvae of rice stem borer are transferred, soon after they hatch, to the test plants with the help of a soft brush. The extent of damage on the test plants is then recorded and compared with that on known susceptible control varieties infested in the same manner. In some cases, seedlings grown in seed boxes are infested by the concerned pest ; resistant seedlings are identified and transferred to pots. After a period of recovery, the plants are reinfested by the concerned pest and further selection is made for resistance. This technique has been used for many insects, *e.g.*, screening of alfalfa for resistance to spotted alfalfa aphid.

It may be pointed out that field and glasshouse screenings are complementary to each other. The resistant plants/lines identified through glasshouse screening should be evaluated for their resistance in the field under natural infestation, and *vice versa*.

DURABILITY OF RESISTANCE

The term durability of resistance refers to the time required for the appearance of a true resistance-breaking biotype of the concerned insect pest. In case of durable resistance, the time required for emergence of such a biotype is quite long and in some cases such biotypes have not been recorded so far. Examples of durable insect resistance are : resistance to jassids in cotton, resistance to woolly aphid in apples, Hessian fly and stem sawfly resistances in wheat, aphid resistance in raspberry, resistance to spotted alfalfa aphid in alfalfa etc. The emergence of new resistance-breaking biotypes of insect pests is far less frequent than that of new pathotypes of plant pathogens. Even where new resistance-breaking biotypes of insect pests have appeared, they have rarely led to the withdrawal of the concerned resistant varieties from commercial cultivation ; this is quite in contrast to the situation with disease resistance. The appearance of new resistance-breaking biotypes is influenced by a variety of factors, *e.g.*, the insect species, the genetics of resistance, and the features of host plant associated with resistance.

Insect Species. New biotypes of some insects appear more frequently than those of others. For example, appearance of new biotypes is relatively more frequent in nematodes and Hemiptera, particularly aphids, than in moths and butterflies (Lepidoptera), beetles and weevils (Coleoptera), and sawflies (Hymenoptera).

Mode of Inheritance of Resistance. In general, resistance governed by single major gene is less durable than those controlled by two or more oligogenes or polygenes. However, some cases of resistance governed by a single major gene are very durable. On the other hand, a few cases of polygenic resistance are as prone to new resistance-breaking biotypes as monogenic resistance. For example, resistance-breaking biotypes of cabbage aphid are known to overcome the polygenic resistance of rape varieties in New Zealand and England. The longer durability of resistance governed by polygenes and that by two or more oligogenes stems from the fact that such cases generally involve more than one feature of the host plant.

Morphological Characters. Insect resistance associated with morphological and/or anatomical characters of the host plant is generally more durable than the other kinds of resistance. For example, resistance to many pests is associated with hairiness of stem and leaves ; hairiness interfers with oviposition by some insects, and retards the development of larvae by impeding their movement. Overcoming a resistance of this type would require a gross change in the insect affecting its oviposition and larval movement. It is also possible that the genes governing the morphological traits associated with resistance in such cases may be closely linked with genes which affect the development of the concerned pest in other ways as well (antibiosis).

There are some cases of durable resistance associated with biochemical factors as well, *e.g.*, resistance of cotton to several pests

associated with high gossypol content, resistance of maize to European corn borer associated with high DIMBOA content, resistance of rice to stem borer associated with high silica content, and resistance of alfalfa to alfalfa aphid associated with high saponin content.

PREVENTION OF INSECT OUTBREAKS

Although true resistance-breaking biotypes of many insects have been reported, resistant varieties have usually provided a good and dependable control measure for them. The past experience clearly indicates that the appearance of such biotypes is unlikely to reduce substantially the potential usefulness of resistant varieties in the field before many years, and it is very unlikely that they will lead to a complete breakdown of resistance over a wide area. Therefore, breeding for insect resistance will continue to be a rewarding objective in spite of the appearance of resistance-breaking biotypes. The plant breeder, however, may adopt one or more of the following approaches to reduce the chances of appearance of resistance breaking biotypes.

1. Different genes for resistance to a single insect pest may control different mechanisms, *e.g.*, nonpreference, antibiosis and tolerance. Wherever such opportunities exist, the breeder should try to combine two or more genes controlling different mechanisms into a single variety. The resistance of such a variety will be much less likely to be overcome by the insect pest than that due to a single gene.

2. Whenever there is a choice of oligogenic and polygenic resistance, the latter should be preferred as it is generally more durable. However, oligogenic resistance which is known to be durable is far more preferable to polygenic resistance since inheritance of and selection for the former is far more simpler as compared to those for the latter.

3. Some workers have suggested the use of multiline varieties. Each component of a multiline should carry a separate gene for resistance to a given insect pest. The usefulness of multilines would depend on (1) the availability of different resistance genes for an insect, (2) nonavailability of a durable oligogenic or polygenic resistance and (3) the extent of damage caused by the insect pest to justify the effort and the expenditure in developing multiline varieties.

4. Alternatively, varieties having different genes for resistance to an insect pest may be planted in different years in a given area. In this way, a variety having a given resistance gene is planted in the area only after few to many years. As a result of such a rotation, a given gene for resistance is not exposed to the pest for a long enough time to permit the evolution of a resistance-breaking biotype. However, it is generally not feasible to ensure a strict rotation over

a large area, and in many cases such a rotation may not be necessary.

PROBLEMS IN BREEDING FOR INSECT RESISTANCE

Breeding for insect resistance suffers from several problems. Some of the more important of these problems are outlined below.

1. In some cases, breeding for resistance to one insect leads to the susceptibility to another pest. This may be because the feature of host plant associated with resistance to one insect is associated with susceptibility to another insect. For example, glabrous (hairless) strains of cotton are resistant to bollworms, but they are susceptible to jassids.

2. In some cases, breeding for insect resistance reduces the quality of produe or may even make it unfit for consumption. High gossypol content is associated with resistance to bollworm in cotton. Since gossypol is a highly toxic compound, a high gossypol content in the seed is undesirable as cotton seed cake is an important feed for livestock, pigs and chicken.

3. In many cases, genes for insect resistance are available only in the related wild species. Interspecific gene transfers present many problems (Chapter 25), and often there is linkage between the gene for resistance and some undesirable genes. Varieties carrying genes for insect resistance from related wild species are generally lower yielding and their produce is often of inferior quality.

4. Screening for insect resistance is the most critical and difficult step in a breeding programme for insect resistance. This necessitates a close coordination between the breeder and the entomologist. In addition, pathologist, biochemist and physiologist have to be closely involved in an efficient breeding programme. A close and smooth collaboration between workers of such diverse disciplines is an ideal rarely achieved in this country.

5. Breeding for insect resistance is a long-term programme. Therefore, adequate financing over a long period of time with a follow-up action is essential. Often lack of funds leads to the interruption of otherwise promising programmes.

ACHIEVEMENTS

Insect resistant varieties have been developed in many crops in a number of countries of the world. The most striking example of the contribution of an insect resistant variety is the introduction of *Phylloxera*-resistant grape root-stocks from USA into France. In U.S.A., 23 Hessian fly resistant wheat varieties have been developed. These varieties are successfully cultivated in the area of Hessian fly incidence. Wheat variety 'Rescue' has solid stem and is resistant to stem sawfly. This variety was first released in 1946 in Canada and is widely grown in those areas of U.S.A. and Canada

where this pest is a problem. Rescue has a lower yield and poorer quality than some susceptible wheat varieties. Therefore, a rotation of two years Rescue-one year a high yielding susceptible variety keeps the stem sawfly population to an acceptable low level and allows a higher return than that from a continuous cultivation of Rescue.

A barley variety 'Will' developed in U.S.A. is resistant to green bugs. Cultivation of this variety has reduced the green bug population to less than 50%. Alfalfa varieties 'Cody' 'Moapa', and 'Zia' developed in U.S.A. are resistant to spotted alfalfa aphid. Oat variety 'Greta', bred in Belgium, is resistant to stem eelworm. Cotton varieties B 1007, SRTI, Khandwa 2, and DHY 286 evolved in India are resistant to jassids and are successfully grown in the Central India. Insect resistant varieties have been developed in many other crops, *e.g.*, in raspberry for resistance to aphid, in lettuce for resistance to root aphid, in grapes for resistance to *Phylloxera*, in apple for resistance to woolly aphis etc.

BREEDING FOR RESISTANCE TO PARASITIC WEEDS

Some weeds parasitise a number of economically important crop species, and often cause a substantial reduction in their yields. Jowar and bajra are parasitised by a weed called *Striga* in some parts of India, *e.g.*, Maharashtra, and Africa. It grows on the roots of these crops and may lead to severe losses in yields in heavily infested crops. Similarly, solanaceous crops like tobacco, tomato etc. are infested by a parasitic weed called *Orobanche*. *Orobanche* infestation of these crops is encountered in most parts of India. In West Asia and the Mediterranean, *Orobanche* also infects winter-sown chickpea (gram).

A convenient and relatively cheaper way of reducing the losses in crop yields caused by such parasitic weeds is to develop resistant or tolerant varieties of their hosts. Their is considerable evidence to suggest that breeding for such resistant varieties is practically feasible. A *Striga*-resistant variety of jowar, SAR-1, has been developed by ICRISAT, Hyderabad. This variety has shown high levels of resistance to *Striga* in the endemic areas of Maharashtra where *Striga* infestation is a serious problem. Some other jowar lines have shown high levels of resistance to *Striga* in certain parts of Africa. Similarly, four bajra lines developed by ICRISAT show 90 per cent less *Striga* incidence than the check variety. Eleven chickpea lines resistant to *Orobanche* and 170 lines possessing tolerance to this parasite have been identified by ICARDA, Syria. These lines are the likely sources for resistance to *Orobanche* in chickpea breeding programmes.

These examples serve to illustrate vividly the point that resistance to parasitic weeds may be located in the germplasm of the host crops if an extensive and sustained search is conducted. Breeding for resistance to such parasitic weeds may be an important breeding objective in the areas where these weeds cause considerable economic losses, and in few areas this may be as high in priority as that for a major disease or pest of the crop.

SUMMARY

Insect resistance is the ability of certain host strains to produce larger yields of good quality than others at the same level of initial infestation under similar environmental conditions. Insect attacks reduce yield as well as quality of the produce either by sucking the cell sap (aphids, jassids, thrips, mites etc.) or by eating away plant tissues (borers, hoppers, beetles, weevils etc.). Resistance to insects results from (1) nonpreference (plants unattractive or unsuitable for colonization or oviposition), (2) antibiosis (feeding on plants adversely affects the development or reproduction), (3) tolerance (ability of the host to produce more than other varieties at the same level of infestation), and (4) avoidance (escape from infestation). Resistance is associated with certain (1) morphological (leaf and stem hairiness, solid stem, thickness and toughness of plant tissues, colour of plant etc.), (2) physiological (exudates, osmotic pressure of cell sap etc.), and (3) biochemical factors (gossypol, silica, benzyl alcohol, tannin, saponin etc.)

Genetically, resistance may be controlled by (1) one or more major genes (*oligogenic*), (2) *polygenes* or (3) *plasmagenes*. Resistance may be completely or partially dominant or recessive to susceptibility. The *sources of resistance* are (1) cultivated varieties, (2) germplasm collections, and (3) related wild species. *The methods of breeding* for insect resistance are (1) introduction, (2) selection, and (3) hybridization (pedigree and backcross). Screening for insect resistance may be done in the field or the glasshouse ; both the screening methods have their merits and demerits

The *durability of resistance* depends mainly on the appearance of true resistance-breaking biotypes. The appearance of resistance-breaking biotypes depends on (1) the insect species, (2) the genetics of resistance, and (3) the mechanism of resistance. Resistance of a variety may be prolonged by (1) bringing in a single variety two or more major genes governing different mechanisms of resistance, (2) using polygenic resistance, (3) gene deployment, and (4) using multilines. Many insect resistant varieties have been developed in various crops in different countries which are successfully grown in the insect prone areas.

Some parasitic weeds infest jowar, bajra, chickpea, tobacco, tomato and several other crops. Some varieties of the host crops are resistant or tolerant to these parasitic weeds. Resistant varieties of the host crop may be developed through breeding in the endemic areas for these weeds.

QUESTIONS

1. Explain the various mechanisms of insect resistance in crop plants with the help of suitable examples.
2. Describe the different types of insect resistance with the help of suitable examples.
3. Write short notes on the following : (*i*) genetic variability in insect pests, (*ii*) inheritance of insect resistance, (*iii*) true resistance-breaking biotypes, (*iv*) durable resistance, (*v*) losses due to insect attack.
4. Differentiate between : (*i*) antibiosis and antixenosis, (*ii*) resistant and susceptible types, (*iii*) oligogenic and polygenic resistance, (*iv*) insect biotype and pathotype, (*v*) pest resistance and pest avoidance.
5. Describe the various sources of insect resistance in crop plants, and discuss their relative usefulness in breeding for insect resistance.
6. Define durable resistance. Discuss the various factors affecting the durability of resistance. Describe the approaches to increase the durability of insect resistance of a variety.
7. An unadapted variety has the desired insect resistance which is governed by a recessive gene. Describe the breeding method for exploitation of this resistance.

8. Describe briefly the various screening techniques for insect resistance, and discuss their merits and demerits.
9. 'Genetic resistance is the most desirable and useful method of controlling a pest'. Comment.

Suggested Further Reading

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CHAPTER 23

Mutations in Crop Improvement

Mutation is a sudden heritable change in a characteristic of an organism. This definition requires that the change in the characteristic be heritable, but it does not state the genetic basis of the heritable change. Clearly, a mutation (as defined above) may be the result of a change in a gene, a change in chromosome(s) that involves several genes or a change in a plasmagene (genes present in the cytoplasm, *e.g.*, in chloroplast, mitochondria etc., which have circular naked DNA as chromosomes). Mutations produced by changes in the base sequences of genes (as a result of base pair transition or transversion, deletion, duplication or inversion etc.) are known as *gene* or *point mutations*. Gene mutations can be easily and clearly shown by fine genetic analysis techniques available with microorganisms. Some mutations may be produced by changes in chromosome structure, or even in chromosome number ; they are termed as *chromosomal mutations*. Gross chromosomal changes, *e.g.*, changes in chromosome number, translocations, inversions, large deletions and duplications, are detectable cytologically under the microscope. But small deletions and duplications can rarely be detected, and would be considered as gene mutations. This is particularly so in higher organisms where the techniques of genetic analysis are not yet as refined as those in the case of microorganisms. Thus what we refer to as gene mutations in plants is likely to include a fair number of small chromosomal changes. In clonal crops, mutations may even include gross changes in chromosome structure, sometimes even in number, unless cytological analyses are performed. *Therefore, in this chapter, the word mutation would be used without a reference to the change in gene or chromosome (but easily detectable chromosome changes are not included) because in most of the cases the site of change is not known.* When the mutant character shows cytoplasmic or extranuclear inheritance, it is known as *cytoplasmic mutation*. Another term, *bud mutation* or *somatic mutation*, is used to denote mutations occurring in buds or somatic tissues which are used for propagation, *e.g.*, in clonal crops.

HISTORICAL ACCOUNT

The term mutation was introduced by Hugo de Vries in 1900. But mutations were known to occur in animals and plants much

before this time. For example, a short-legged sheep was discovered by an English farmer in the 18th century ; this sheep was used to establish a breed named Ancon. Mutagenic action of X-rays was discovered by Muller in 1927 on *Drosophila*, and in 1929 by Stadler in barley (*H. vulgare*) and maize (*Z. mays*) ; Muller was awarded the Nobel Prize in 1946 in recognition for this work. In 1946, Auerbach and Robson showed that nitrogen mustards produced mutations in *Drosophila*. Subsequently, a number of chemicals with mutagenic action were described. Immediately after Muller's discovery in 1927, mutation breeding programmes were initiated in Sweden, U.S.S.R. and Germany. The Swedish mutation breeding programme was started in 1929 by Nilsson-Ehle and subsequently continued by Ake Gustafsson and coworkers. The Swedish programme is perhaps the most extensive covering several crop species, and much valuable information has come out of the programme. Mutation breeding attracted considerable attention during 1950s and 1960s, and several countries took up research projects in mutation breeding. In the early days of mutation breeding, it was felt that mutation breeding would revolutionise plant breeding, and the breeder would be able to create new genes at will. Indeed, this had been the case with the other special techniques in plant breeding, *e.g.*, polyploidy, *in vitro* techniques, etc. But as knowledge accumulated, it became clear that although mutation breeding has a definite role to play, it is not the magic wand which would create what you wanted and when you wanted.

SPONTANEOUS AND INDUCED MUTATIONS

Mutations occur in natural populations (without any treatment by man) at a low rate ; these are known as *spontaneous mutations*. The frequency of spontaneous mutations is generally one in 10 lacs, *i.e.*, 10^{-6}, but different genes may show considerably different mutation rates. For example, *R* locus in maize mutates at a frequency of 4.92×10^{-4}, *Su* at 2.4×10^{-6}, while *Wx* appears to be highly stable. Spontaneous mutation rates of genes may be considerably affected by the genetic background ; some *mutator genes* may promote mutation of other genes, *e.g.*, gene *Dt* (dotted) on chromosome 9 in maize increases the mutation frequency of *a* (colourless aleurone) to *A* (coloured aleurone), which is present on chromosome 3. Mutations may be artificially induced by a treatment with certain physical or chemical agents ; such mutations are known as *induced mutations*, and the agents used for producing them are termed as *mutagens*. The utilization of induced mutations for crop improvement is known as *mutation breeding*.

The available evidence indicates that induced mutations rarely produce new alleles ; they produce alleles which are already known to occur spontaneously or may be discovered if an extensive search were made. It is reasonable to say that induced mutations are comparable to spontaneous mutations in their effects and in the

variability they produce. But the induced mutations have a great advantage over the spontaneous ones : they occur at a relatively higher frequency so that it is practical to work with them. Mutations have certain general characteristics ; those that concern us the most are summarised below.

1. *Mutations are generally recessive*, but dominant mutations also occur.
2. *Mutations are generally harmful to the organism.* Most of the mutations have deleterious effects, but a small proportion (ca. 0.1 per cent) of them are beneficial.
3. *Mutations are random, i.e.*, they may occur in any gene. However, some genes show higher mutation rates than others.
4. *Mutations are recurrent*, that is, the same mutation may occur again and again.
5. Induced mutations commonly show pleiotropy, often due to mutations in closely linked genes.

EFFECTS OF MUTATION

Generally, mutations have harmful effects on organisms. Usually they reduce viability of the individuals that carry them. Based on their effect on viabilityt , mutations are classified into four groups : lethal, sublethal, subvital and vital.

Lethal. Lethal mutations kill each and every individual that carries them in the appropriate genotype. Dominant lethals, therefore, cannot be studied because they cannot survive even in the heterozygous state. Thus, of necessity, we have to consider only recessive lethals ; that is, genes recessive in their lethal action. Recessive lethals would kill the individuals that carry them in the homozygous state, *e.g.*, albina chlorophyll mutations.

Sublethal And Subvital. Sublethal and subvital mutations reduce viability, but they do not kill all the individuals carrying them. Sublethals kill more than 50 per cent of the individuals, while subvitals kill much less than 50 per cent. *A vast majority of mutations are lethals, sublethals and subvitals ; these are of no value in crop improvement,* although some of them may be of academic interest.

Vital. Vital mutations do not reduce viability of the individuals carrying them. *Obviously, crop improvement can utilize only such mutations. Vital mutations occur in a much lower frequency as compared to the other three types.*

MUTAGENS

Agents used for the induction of mutations are known as mutagens. Mutagens may be different kinds of radiations (*physical*

mutagens) or certain chemicals (*chemical mutagens*). A detailed discussion of various properties of the mutagens is beyond the scope of this book. But some of the important properties of different mutagens will be summarised here. The different mutagens may be grouped as follows.

A. Physical mutagens (all of them are various kinds of radiations)
   1. Ionising radiations
      (a) Particulate radiations, *e.g.*, α-rays (DI), β-rays (SI), fast neutrons* (DI), and thermal neutrons (DI).
      (b) Nonparticulate radiations (electromagnetic radiations), *e.g.*, *X*-rays* (SI), and γ-rays* (SI).
   2. Nonionising radiations, *e.g.*, ultraviolet radiation (UV).

B. Chemical mutagens
   1. Alkylating agents, *e.g.*, sulphur mustards, nitrogen mustards, epoxides, ethylene-imines, (*e.g.*, ethylene imine or EI)*, sulphates and sulphonates (*e.g.*, ethylmethane sulphonate or EMS*, methylmethanesul phonate or MMS), diazoalkanes, nitroso compounds *e.g.*, N′-methyl-N-nitro-N-nitroso-guanidine or MNNG).
   2. Acridine dyes, *e.g.*, acriflavine, proflavine, acridine orange, acridine yellow, ethidium bromide.
   3. Base analogues, *e.g.*, 5-bromouracil, 5-chlorouracil.
   4. Others, *e.g.*, nitrous acid, hydroxyl amine, sodium azide*.

[*denotes that these agents are commoly used in mutation breeding. DI denotes densely ionising and, SI denotes sparsely ionising radiations.]

Beta-Rays. Beta-rays are high energy electrons produced by the decay of radioactive isotopes, *e.g.*, ^{3}H, ^{32}P, ^{35}S etc. High energy electrons are showed down by positively charged molecules in tissues, and they have very little penetrating power as compared to X-rays. Beta-rays transfer their energy to electrons of the atoms in their path causing these electrons to fly away from their orbit leaving the nucleus positively charged ; this is known as *ionisation.* When the amount of energy transferred to an electron is not sufficient to cause ionisation, the electron is pushed to an outer orbit representing a higher level of energy, thus producing *excitation.* Electrons are easily deflected by atoms in their path, hence they move in a zig-zag line. After their energy is spent, electrons attach to an atom making it negatively charged. Beta-rays may interact with the nuclei of atoms to produce electromagnetic radiations similar to X-rays. *Electrons liberated as a result of ionisation also produce ionisation and excitation, that is, they behave like beta-rays.*

Alpha-Rays. The alpha particles making up alpha-rays have two protrons and two neutrons each ; thus the alpha-particles have double positive charge. Alpha-particles are produced by fission of radioactive isotopes of heavier elements. Since they are heavy

particles, they move in a straight line. Alpha-particles have a strong attraction for electrons and pull them away from the nuclei of atoms in their path. Alpha-rays produce both ionisation and excitation. After losing energy, each alpha-particle captures two electrons and produces an atom of Helium. As the α-particles move away from their source, they slow down and produce dense ionisation. Alpha-particles are much less penetrating than neutrons and even beta-rays.

Fast and Thermal Neutrons. *Fast Neutrons* are produced in cyclotrons or atomic reactors as a result of radioactive decay of heavier elements. The velocity of fast neutrons is reduced by graphite or heavy water to generate *thermal or slow neutrons.* Neutrons are uncharged particles, and are highly penetrating in biological tissues. They are not repelled by nuclei of atoms, and move in a straight line. They do not cause ionisation directly. Ionisation is produced by (1) *elastic scattering*, in which nuclei of atoms are kicked away by the neutron ; these nuclei then cause ionisation, and (2) *production of gamma-rays* as thermal neutrons are captured by atomic nuclei, which then become unstable and give off gamma-rays. Fast and thermal neutrons are densely ionising radiations.

X-rays and Gamma Rays. X-rays and gamma-rays are nonparticulate electromagnetic radiations with a wavelength of 10^{-11} to 10^{-7} cm (0.001-10Å or 10^{-4}—1 nm). These are high energy radiations and consist of photons, *i.e.*, small packets of energy. The physical properties and the biological effects of X-rays and gamma-rays are similar, but they differ in the source of their origin. X-rays are produced by X-ray tubes, while gamma-rays are produced by radioactive decay of certain elements, *e.g.*, radium, ^{14}C, ^{60}Co etc. ^{60}Co is the common source of gamma-rays used for biological studies. X-rays are often referred to as *hard* (0.1-0.001Å) or *soft* 10-1Å) depending upon their wavelength. X-rays and gamma-rays are highly penetrating and sparsely ionising. The electro-magnetic radiations produce the following effects.

Photoelectric Effects. Low energy photons transfer all their energy to individual electrons which are kicked off as high energy electrons (e^-) from their orbit producing ionisation (Fig. 23.1). These high energy e^- produce secondary ionisations which are of greater significance than the primary ones.

Compton Scattering. A high energy photon transfers a part of its energy in kicking away an e^- from its orbit producing ionisation. The wavelength of a photon becomes increasingly longer as it loses energy in repeated ionisations (Fig. 23.1).

Pair Production. A high energy photon passing close to the nucleus of an atom may be completely absorbed accompanied with the ejection of a high energy e^- and a high energy position (e^+); the high energy e^- and e^+ produce ionisation and excitation (Fig. 23.1).

Ultraviolet Radiation. Ultraviolet rays have a wavelength of 100 to 3900Å (10-390 nm). UV is present in solar radiation and is produced

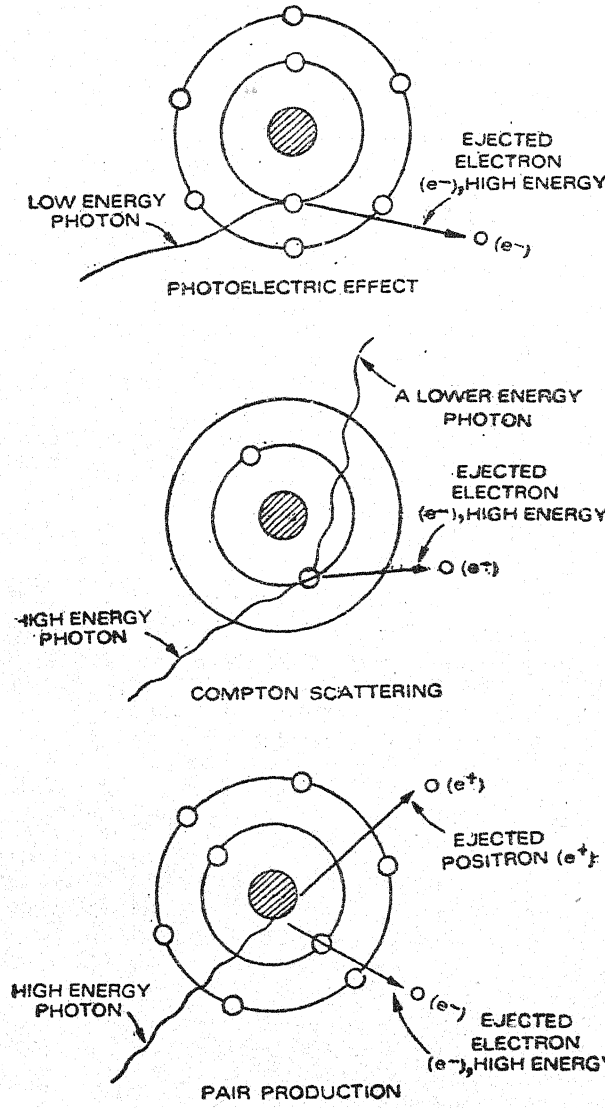


Fig. 23.1. Ion production by X-ray and gamma-ray photons. The large central solid circle represents the nucleus of the atom, small open circles or the periphery denote electrons (e^-), and the particle e^+ is a positron The atom pictured here is an oxygen atom.

by mercury-vapour lamps or tubes. UV is a low energy radiation ; it does not cause ionisation and has a very limited penetrating capacity (usually limited to one or two cell layers). UV rays generally produce dimers of thymine, uracil and, sometimes cytosine present in the same strand of DNA. It also produces addition of a molecule of water to the 5, 6 double bond of uracil and cytosine, which promotes deamination of cytosine. The mutagenic action of UV is most likely due to dimer formation and deamination. The most effective wavelength of UV is 2,540 Å since DNA bases show the maximum absorption at this wavelength.

UV is commonly used in microorganisms since penetration presents no problem in that system. But in higher organisms, poor penetration of UV has limited its use to irradiation of pollen grains (in plants) and of small eggs (*e.g.*, in *Drosophila*). In plants, pollen grains may be irradiated and used for pollination. But the difficulty in collecting large quantities of pollen grains in most of the crop species, except in maize and similar crops, and the limited duration of pollen viability have prevented the use of UV in crop improvement.

MECHANISM OF ACTION OF RADIATIONS

Chemical effects of radiations are direct as well as indirect. In the case of *direct effect*, energy is transferred to a molecule directly by the radiation. *Indirect effect* is mediated by free radical formation ; these radicals transfer their energy to other molecules. The indirect effect is particularly important in the presence of water since ionised water molecules produce free radicals. Excitation has a very low efficiency in producing altered molecules, but sometimes it may cause dissociation of molecules. *The primary effect of radiations is due to ionisation ; the mechanism of ionisation differs to some extent from one radiation to the other.* The chemical effects of radiations may be summarised as under.

Formation of Ion Pairs. An electron is ejected from an atom to produce a positively charged ion. The ejected e^- is captured by another atom to produce a negatively charged ion. Thus a pair of ions (one positively and one negatively charged ion) is produced which is known as ion-pair (Fig 23.2). Oxygen (O_2) is one of the common electron acceptors. *Biologically significant events are considered to be produced by ion-pairs.*

Formation of Free Radicals. Free radicals have a very short life of 10^{-10} seconds. *Free radicals are electrically neutral molecules with an unpaired electron in their outer orbit. A fair portion of genetic effects of radiations are the results of the free radicals produced by irradiation.* Production of free radicals from water is illustrated in Fig. 20.3. An e^- is ejected from the oxygen atom of H_2O, which is captured by one H atom of another water molecule producing an ion-pair. In the presence of two other water molecules, the ion-pair dissociates and produces two free radicals.

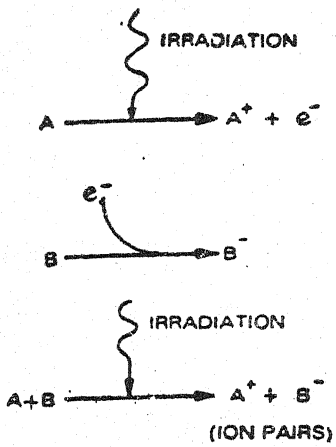


Fig. 23.2. Production of ion-pairs by high energy radiations.

H^0 and OH^0 are free radicals which are highly reactive. They produce H_2O_2 (Fig. 23.4) and organic peroxides, which are highly reactive and are thought to be responsible for a good portion of the biological effects of radiations.

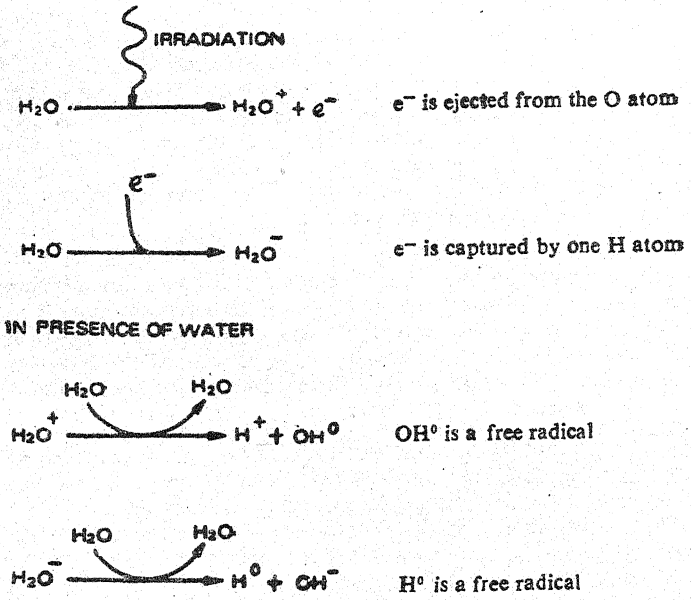


Fig. 23.3. Production of free radicals by high energy radiations, using water molecules as an example.

Genetic effects of radiations result from their effect on DNA. These effects include, change in a base, *e.g.*, deamination, loss of a base, breaking of hydrogen bonds in DNA, single and double strand breaks in DNA and cross-linking of strands. Pyrimidines are more radiosensitive, *i.e.*, sensitive to radiation damage. DNA present in the chromatin is much more protected than naked DNA because of the protein coating. Deamination of

Free radicals are removed as follows,

$$H^0 + OH^0 \rightarrow H_2O$$
$$H^0 + H^0 \rightarrow H_2$$
$$OH^0 + OH^0 \rightarrow H_2O_2$$

Presence of O_2 increases the yield of H_2O_2,

$$H^0 + O_2 \rightarrow HO_2^0$$
$$HO_2^0 + HO_2^0 \rightarrow H_2O_2 + O_2$$

H_2O_2 is destroyed as follows,

$$H_2O_2 + H^0 \rightarrow H_2O + OH^0$$

Fig. 23.4. Production of H_2O_2 from free radicals

bases and intrastrand dimerisation may lead to changed base pairing producing changes in the base sequence of DNA, that is, gene mutation.

MECHANISM OF ACTION OF CHEMICAL MUTAGENS

The mechanism of action of chemical mutagens varies to a great extent from one group of chemical mutagens to another. It is not possible to explain the action of all the classes of chemical mutagens in this text. A broad general summary is presented instead. The changes in DNA molecule produced by mutagens may be mutagenic or inactivating. *Mutagenic changes* do not prevent replication, produce changes in one or more nucleotides and do not induce chromosomal aberrations. They result from changes in hydrogen bonding properties of bases or from mistakes in base pairing during DNA replication. The events at the molecular level are either substitution (transition and transversion), deletion or insertion of bases. Substitution of bases generally has a less drastic effect than deletion or insertion. Deletion and insertion often produce frame-shift mutations since they usually change the sequence of bases in all the codons that follow the point of deletion or insertion. *Inactivating alterations* prevent DNA replication across the altered site, induce chromosome breaks and chromosome mutations. The inactivating alterations include removal of bases, dimer formation, cross-linking of the two DNA strands, and single or double strand breaks. *Most of the inactivating alterations of DNA are repaired by cellular enzymatic repair mechanisms.*

The mechanism of action of alkylating agents is described in some detail. The alkylating agents have one, two or more reactive groups that react with DNA, hence they are known as mono-di-or

polyfunctional, respectively. Polyfunctional, sometimes even monofunctional, agents cause extensive cross-linking of DNA, chromosome breaks, chromosome mutations and gene mutations. The action of alkylating agents may be summarised as under.

1. *Reaction with PO_4* in the DNA PO_4-sugar backbone producing semistable triesters. This may cause backbone breaks.
2. *Reaction with ring nitrogen*, particularly in guanine with the N at position 7, rarely with N in cytosine. Alkylation (reaction with an alkyl group) of the ring N leads to the removal of a base, *i.e.*, depurination (since guanine is a purine) and may lead to backbone breaks.
3. *Transition of G : C to A : T* may result due to mistakes in base pairing following alkylation of guanine.

PROCEDURE FOR MUTATION BREEDING

Treating a biological material with a mutagen in order to induce mutations is known as *mutagenesis*. Exposure of a biological material to one of the radiations (X-rays, gramma-rays etc.) is known as *irradiation*. When mutations are induced for crop improvement, the entire operation of the induction and isolation of mutants is termed as *mutation breeding*. A mutation breeding programme should be clearly planned and should be large enough with sufficient facilities to permit an effective screening of large populations. The various steps involved in mutation breeding are briefly discussed below.

Objectives of the Programme. *A mutation breeding programme should have well defined and clear-cut objectives.* If the experimenter starts a mutagenesis programme just with the hope that he will discover something good, he is most likely wasting his time and resources. This is because the ratio of beneficial to useless mutations is very small (1 in 800 mutations, that is, about 0.1 per cent of mutations), and identifying desirable mutations from the undesirable ones is a very difficult task indeed. Further, if a character governed by oligogenes is to be improved, the procedure for the handling of treated populations would be different from that when a polygenic trait is to be improved.

Selection of the Variety for Mutagen Treatment. *Generally, the variety selected for mutagenesis should be the best variety available in the crop.* This is particularly so when polygenic traits are to be improved. It serves no purpose to isolate desirable mutants in a less adapted inferior variety only to discover that the mutants have no agricultural worth, or that the mutants have to be used in a hybridization programme for transferring the mutant characteristics to a superior variety. In certain situations, however, it may be desirable to isolate mutants in varieties other than the best one. For example, an extensive search is being made for alternative dwarfing genes in cereals, particularly in wheat and rice (*O. sativa*). In this case, the

dwarf and semidwarf mutants would have to be isolated from a tall variety, which obviously would not be the best variety.

Part of The Plant to Be Treated. Seeds, pollen grains or vegetative propagules (buds and cuttings) may be used for mutagenesis. Which plant part should be used for mutagen treatment depends primarily on whether the crop is sexually or asexually propagated and on the mutagen to be used. In sexually propagated crops, seeds are the most commonly used plant part. Pollen grains may be used, but they are infrequently used because (1) it is difficult to collect large quantities of pollen grains in most of the crop species, (2) hand pollination (with treated pollen) is difficult, and (3) pollen survival is relatively short. In case of clonal crops, buds or cuttings are used for mutagenesis. Radiations (except UV) are suitable for use with all the three plant parts and even with the whole plants. Whole plants are generally irradiated during the flowering stage so that it is equivalent to the irradiation of pollen grains and egg cells. However the treatment of whole plants requires special facilities (a gamma garden) and is possible in a few places only. Chemical mutagenes are best used with seeds, but some workers have used them with vegetative propagules as well.

Dose of The Mutagen. Mutagen treatments reduce germination, growth rate, vigour and fertility (pollen as well as ovule). There is considerable killing of plants during the various stages of development after mutagen treatment ; thus survival is reduced considerably. Mutagens generally induce a high frequency of chromosomal changes and mitotic and meiotic irregularities. Usually, the damage increases with the mutagen dose, but it may not necessarily be proportional. *An optimum dose is the one which produces the maximum frequency of mutations and causes the minimum killing.* Many workers feel that a dose close to LD_{50} should be the optimum. LD_{50} is that dose of mutagen which would kill 50 per cent of the treated individuals LD_{50} varies with the crop species and with the mutagen used. A preliminary experiment is generally conducted to determine the suitable mutagen dose. *In general, an overdose is likely to kill too many treated individuals, while an underdose would produce too few mutations.*

Dose of the mutagen may be varied by varying the intensity or the treatment time. In case of radiations, intensity may be varied by changing the radiation source or by changing the distance from the radiation source of the material being irradiated. Intensity in the case of chemical mutagens may be varied by changing the concentration of mutagens.

Giving the Mutagen Treatment. The selected plant part is exposed to the desired mutagen dose. In case of irradiation, the plant parts are immediately planted to raise M_1 plants from them (pollen grains are used for pollination). In case of chemical mutagens, seeds are usually presoaked for a few hours to initiate metabolic activities, exposed to the desired mutagen and then washed in running tap water to remove the mutagen present in the seeds. The treated seeds

are immediately planted in the field to raise M_1 generation. M_1 is the generation produced directly from the mutagen-treated plant parts without a recourse to sexual or asexual reproduction. But when pollen grains are treated, the generation resulting from the seeds produced by the treated pollen grains would be the M_1 generation. M_2, M_3, M_4 etc. are the subsequent generations derived from M_1, M_2, M_3 etc. plants through selfing or clonal propagation.

Handling of The Mutagen-Treated Population. Treatment of seeds and vegetative propagules commonly produces chimeras. A *chimera* is an individual with one genotype in some of its parts and another genotype in the others. Shoot-tip meristem usually has two functional layers : the *outer layer*, giving rise to epidermis and a part of leaf mesophyll, and the *inner layer* producing the rest of the plant tissues including reproductive organs. When the entire outer or inner layer is affected, the chimera is known as *periclinal chimera* (*inner periclinal* or *outer periclinal* depending upon the layer affected), while in a *sectorial chimera* only a part of the inner or the outer layer is affected (*inner sectorial* and *outer sectorial*, respectively) (Fig. 23.5)

In sexually reproducing species, only the inner chimera (periclinal or sectorial) will be transmitted to the next generation ; outer chimeras will not be recovered since this layer does not contribute to the production of gametes. In clonal crops, however, both outer and inner chimeras can be utilized either as periclinal chimeras (outer or inner) or by producing homogeneous individuals through sexual reproduction (only if the inner layer is affected), tissue culture or other horticultural manipulations, *e g.*, wounding etc., which induce production of adventitious shoot buds (both inner and outer chimeras utilized). Sectorial chimeras are unstable in clonal crops and have to be made periclinal through successive clonal propagation and selection for stability.

Mutations usually occur in small sectors of the meristem and as a result only a part of the plant is affected. One or more sexual or clonal generations coupled with selection are necessary to obtain a stable mutant phenotype. Mutant alleles are generally recessive, but some dominant mutations may also occur. *In case of sexually reproducing crops, mutations breeding utilizes both recessive and dominant mutations and, in addition, excellent opportunities exist for mutation breeding for polygenic traits. Mutation breeding in clonal crops primarily depends on dominant mutations ; recessive mutations may also be utilized provided the clone used for mutagen treatment was heterozygous for the gene in question.* For example, if recessive mutant allele *a* is to be useful in a clonal crop, the clone has to have the genotype *Aa*. Such situations are however rare ; more frequently, the mutants useful in the improvement of clonal crops are dominant mutations and may include changes in chromosome structure or even number.

The following discussions are based on sexually reproducing species, more particularly self-pollinated species. Since *dominant mutations* are able to express themselves in heterozygous state,

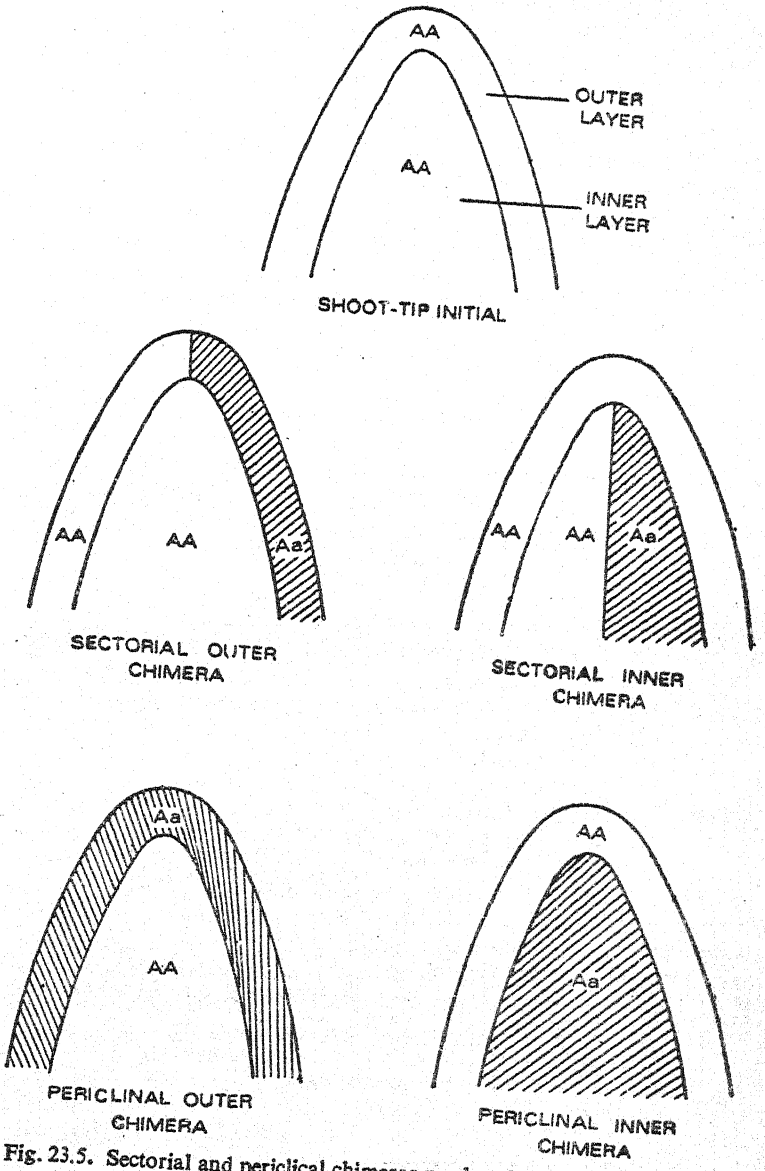


Fig. 23.5. Sectorial and periclical chimeras produced by mutagenesis. The mutant allele is recessive (*a*).

mutant plants are selected in M_1. M_2 and M_3 are raised and homozygous mutants are selected. Selection for *recessive mutations*, however, can be taken up in M_2 only, but the mutant allele will be homozygous in the M_2 itself. Selection for polygenic traits is delayed till M_3 generation, and is based on individual plant progenies rather than on individual plants. A generalised scheme for handling the mutagen-treated populations for oligogenic and polygenic traits is outlined below.

Mutations in Oligogenic Traits. The handling procedure described here (Fig. 23.6) is based on the selection for a recessive mutant allele.

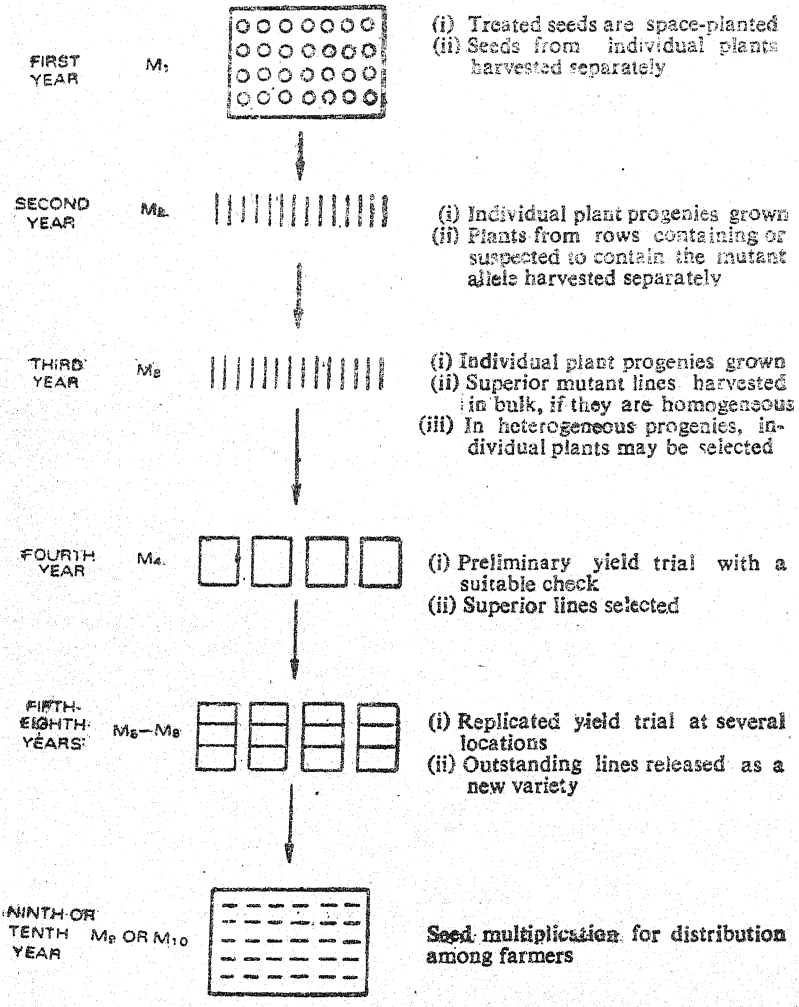


Fig. 23.6. A generalised scheme for mutation breeding for an oligogenic trait ; the mutant allele is recessive.

1. M_1. Several hundred (500 or more) seeds are treated with a mutagen and are space-planted. M_1 plants will be chimeras for the mutations present in heterozygous state. About 20 seeds from each M_1 spike (in case of cereals) or plant are harvested separately to raise the M_2 progeny rows.

2. M_2. About 2,000 progeny rows are grown. Careful and regular observations are made on the M_2 rows. But only distinct mutations are detected in M_2 because the observations are based on single plants. All the plants in M_2 rows suspected of containing new mutations are harvested separately to raise individual plant progenies in M_3. If the mutant is distinct, it is selected for multiplication and testing. However, most of the mutations will be useless for crop improvement. Only 1-3 per cent of M_2 rows may be expected to have beneficial mutations.

3. M_3. Progeny rows from individual selected plants are grown in M_3. Poor and inferior mutant rows are eliminated. If the mutant progenies are homogeneous, two or more M_3 progenies containing the same mutation may be bulked. Mutant M_3 rows are harvested in bulk for a preliminary yield trial in M_4.

4. M_4. A preliminary yield trial is conducted with a suitable check, and promising mutant lines are selected for replicated multilocation trials.

5. M_5-M_8. Replicated multilocation yield trials are conducted. The outstanding line may be released as a new variety.

Mutations in Polygenic Traits. There is considerable evidence that mutations are induced in polygenic traits and that there is genetic gain under selection. Mutagenesis does produce genetic variation in polygenic traits ; mutant lines superior to the parent variety for polygenic traits including yield have been isolated from mutagen-treated populations through selection. A generalised scheme for the improvement of polygenic traits through mutation breeding is outlined below (Fig. 23.7).

1. M_1 *and* M_2. M_1 and M_2 are grown in the same way as in the case of oligogenic traits. In M_2, vigorous, fertile and normal looking plants that do not exhibit a mutant phenotype are selected and their seed is harvested separately to raise M_3 progeny rows.

2. M_3. Progeny rows from individual selected plants are grown. Careful observations are made on M_3 rows for small deviations in phenotype from the parent variety. Inferior rows are discarded. Few rows may be homogeneous and would be harvested in bulk. Selection is done in M_3 rows showing segregation ; a majority of M_3 rows would show segregation.

3. M_4. Bulked seed from homogeneous M_3 rows may be planted in a preliminary yield trial with a suitable check ; superior progenies are selected for replicated multilocation yield trials. Individual plant progenies from M_3 are critically observed. Progenies showing segregation may be subjected to selection only if they are promising.

Superior homogeneous progenies are harvested in bulk for preliminary yield tests in M_5.

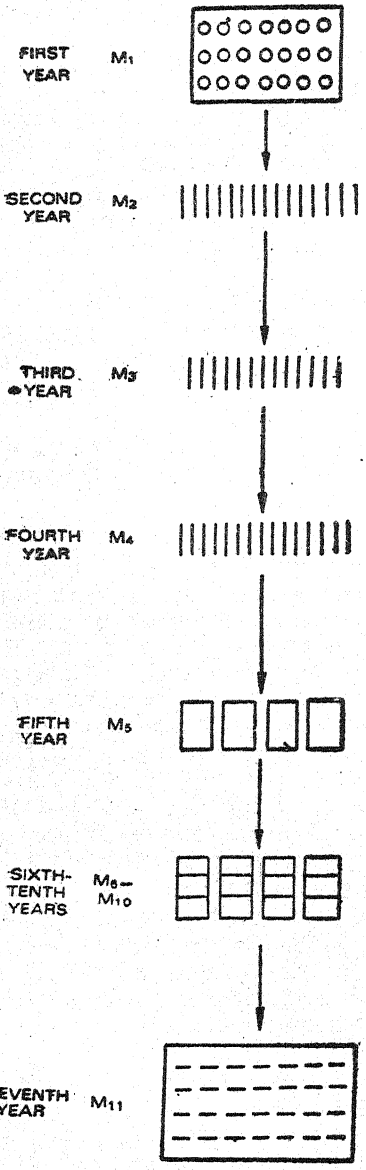


Fig. 23.7. A generalised scheme for mutation breeding for polygenic traits.

4. M_5-M_9. Preliminary yield trials and/or multilocation trials are conducted depending upon the stage when the progenies become homogeneous. Outstanding progenies may be released as new varieties.

Precautions. In mutation breeding programmes, the following two precautions must be carefully observed. *First*, outcrossing with other varieties must be avoided in the M_1, since M_1 plants show considerable male sterility. Therefore, prevention of outcrossing is necessary even in self-polinated species. This may easily be attained by isolation. *Second* precaution relates to avoiding of mechanical mixtures just as in the case of purelines. If proper care is not taken, the breeder may isolate mutations with surprising similarity to known genotypes or varieties (but, in fact, these so called mutants are only mechanical mixtures).

RECURRENT IRRADIATION

In case of recurrent irradiation, several successive generations are subjected to irradiation treatment. Chemical mutagens may also be used for recurrent treatment, but are not commonly used. For treatment, a random sample of seeds from M_1 plants may be taken. Alternatively, one or a few seeds from each M_1 plant may be used for treatment. The dose of mutagen used for recurrent treatment is generally lower than that used for single treatments. After 4-5 generations of recurrent irradiation, the material is handled as in the case of single treatment.

It is expected that recurrent irradiation would accumulate mutations in the population. But there is little evidence to suggest that recurrent irradiation is a profitable scheme ; generally workers have used single treatments in preference to recurrent treatments.

GAMMA-GARDEN

Gamma-garden is an area subjected to gamma-irradiation. This area is enclosed by thick-high walls to protect the plants and animals outside this area from radiation damage. *The purpose of a gamma-garden is to irradiate whole plants during different stages of growth and for varying durations.* The source of radiation is located in the centre of the gamma-garden, which is usually circular in outline. The intensity of radiation decreases as one moves away from the source of radiation. The area around the radiation source is divided into a number of concentric circles representing varying intensities of radiation. Plants are placed in a suitable position in the gamma-garden, which depends upon the growth habit of the species and the intensity of radiation required. The gamma-ray source may also be used for the irradiation of seeds etc.

The first gamma-garden was built in Long Island near New York, U.S.A. The first gamma-garden in India was installed in Calcutta at the Bose Research Institute in 1959. A little later, another gamma-garden was built in New Delhi at the Indian Agricultural Research Institute (IARI) in 1960. A gamma-garden has also been established at the Bhabha Atomic Research Centre, Trombay. The IARI gamma-garden has an area of 3 acres, and is surrounded by a wall 12 feet high and 3 feet thick. This brick wall is, in turn, enclosed with a barbed wire fence. The gamma-ray source consists of 6 g of ^{60}Co in form of small pellets ; the ^{60}Co pellets are sealed in an aluminium capsule. The strength of the ^{60}Co source is 200 Curies. The aluminium capsule is housed in a lead container, since lead is not penetrated by gamma-rays or X-rays. When irradiation is to be done, the lid of the lead container is lifted ; the aluminium capsule containing the ^{60}Co pellets is also raised simultaneously. After irradiation, the aluminium capsule is lowered into the lead container and the lid of the container is closed enclosing the ^{60}Co aluminium capsule in the lead container. The lead container is opened and closed by a remote control device. The present state of the gamma-gardens in the country is quite discouraging and most of them are not in operation.

DIRECTED MUTAGENESIS

It has been a long cherished goal of the workers in mutation breeding to control the direction of induced mutations, *i.e., directed mutagenesis*. Ideally, directed mutagenesis implies the induction of only the desired mutation(s) at a high frequency. But in practice, even an appreciable increase (say 10 to 100 times) in the frequency of desired/desirable mutations would be highly useful. Directed mutagenesis was sought to be achieved by using different mutagens singly as well as in combination, and by varying the internal and/or external environments before, during or after the mutagen treatments. Many of these factors have been able to change the frequency of mutations and, to a small extent, the types of induced mutations as well. For example, irradiation of seeds having a lower moisture content (3-5 per cent) greatly increases radiosensitivity and mutation frequency. In case of chemical mutagens, the duration of presoaking of seeds before mutagen treatment considerably affects the frequency of mutations recovered ; generally a longer presoaking (upto a certain limit) produces a higher frequency of mutations. These are, notable achievements, but the frequency of desirable mutations is rarely increased, and it remains approximately 1 out of every 800 mutations induced or so. Thus at the present time, the type of mutations induced by mutagen treatments is beyond experimental control. The breeder has to sort out what he wants from a lot of undesirable, although often fancy, mutations.

APPLICATIONS OF MUTATION BREEDING

Mutation breeding has been used for improving both oligogenic as well as polygenic characters. Mutagenesis has been used to improve morphological and physiological characters, disease resistance and quantitative characters including yielding ability. The various applications of mutation breeding may be briefly summarised as under.

1. *Induction of desirable mutant alleles which may not be present in the germplasm or that may be present but may not be available to the breeder due to political or geographical reasons.* To some extent, mutation breeding relieves the complete dependence of breeders on the natural germplasm. But it should be remembered that mutation breeding cannot minimise the necessity of germplasm collections ; it only serves as a useful supplement to the available germplasm.
2. *It is useful in improving specific characteristics of a well adapted high yielding variety.* This is particularly so in the case of clonal crops due to their highly heterozygous nature ; in such a case, mutagenesis is the only method available to improve the specific characteristics of clones without changing the genetic make up.

   In self-pollinated species, mutagenesis is useful in improving the specific characteristics of otherwise adapted and superior varieties. However, in such species mutagenesis may not be simpler or quicker than the standard backross procedure, if the characteristic is available in a variety. This is more so because the desirable mutations are often associated with undesirable side effects due to other mutations, chromosomal aberrations, sterility etc. One or few backcrosses with the parent variety may be necessary to bring the desirable mutant allele in an acceptable genetic background.
3. *Mutagenesis has been successfully used to improve various quantitative characters, including yield.* Several varieties have been developed by this techique. However, there is no critical comparison available to show that the same improvement would not have been brought about by the conventional hybridization programmes.
4. *F_1 hybrids from intervarietal crosses may be treated with mutagens in order to increase genetic variability by inducing mutations and to facilitate recombination of linked genes.* There is some evidence that mutagen treatment of the F_1 gives a relatively wider spread (in frequency distribution) in the F_2. Some of the additional variability in F_2 is undoubtedly due to an enhanced recombination between linked genes. But the method has not been widely used in mutation breeding.
5. *Irradiation of interspecific (distant) hybrids has been done to produce translocations. This is done to transfer a chromosome segment* carrying a desirable gene from the alien chromosome

to the chromosome of a cultivated species. This illustrates another application of irradiations in crop improvement, *but this does not constitute mutation breeding.*

LIMITATIONS OF MUTATION BREEDING

The experience with mutation breeding has brought out certain limitations of the technique. These limitations are summarised as under.

1. The frequency of desirable mutations is very low, about 0.1 per cent of the total mutations. Therefore, large M_2 and subsequent populations have to be grown and carefully studied. This involves considerable time, labour and other resources.
2. The breeder has to screen large populations to select desirable mutations. Therefore, efficient, quick and inexpensive selection techniques are required to screen large populations. Mutation breeding is more easily applied to such characters where quick screening techniques are available, *e.g.*, disease resistance. But in the case of characters where elaborate tests are required, *e.g.*, quality characteristics, mutation breeding is virtually impractical. For this reason, mutation breeding has been more successful with those characteristics where the mutant phenotype is distinct and easily detectable.
3. Desirable mutations are commonly associated with undesirable side effects due to other mutations, chromosomal aberrations etc. The mutant line often has to be backcrossed to the parent variety to remove these defects. This increases the time requirement of mutation breeding programmes and involves additional labour, time and expenditure.
4. Most of the mutations are recessive. Detection of recessive mutations is almost impossible in clonal crops and is difficult in polyploid species. Consequently, in polyploid species larger populations have to be grown and larger doses of mutagen have to be applied. Mutagenesis has been most commonly applied to diploid species that reproduce sexually, particularly in self-pollinated species.

ACHIEVEMENTS

More than 337 varieties have been produced as a result of mutagenesis programmes in different countries of the world. Mutant varieties have been developed in cereals, oilseeds, pulses, millets, vegetables, fruit trees etc., but wheat, barley and rice account for about 50% of mutant varieties in all the crops. (Table 23.1). These varieties represent improvements in oligogenic as well as polygenic characteristics. These crop varieties belong to diploid and polyploid sexually reproducing species as well as to clonal crops. The first variety developed from a mutagenesis programme was Primax White Mustard (*Brassica hirta*) released in 1950, followed by Regina II Summer Rape (*B. campestris*) in 1953. Both the varieties were

Table 23.1. A list of mutant varieties of crop plants developed upto 1983 (Based on Mutation Breeding Newsletter upto No. 23, Jan. 1984)

Crop	*Number of mutant varieties*		
	Direct mutants	*Mutants used in hybridization*	*Total*
Cereals			
Wheat (*T. aestivum*)	24	6	30
(*T. turgidum*)	7	8	15
Barley	29	39	68
Rice	44	24	68
Rye	3	0	3
Oat	4	4	8
Millets			
Maize	3	4	7
Bajra	1	1	2
Oilseeds			
Soybean	8	1	9
Groundnut	5	2	7
Rapeseed	5	0	5
Sunflower	1	0	1
Linseed	1	1	2
Castor	2	1	3
Sesamum	1	0	1
White mustard	1	2	3
Pulses			
Mungbean	3	0	
Vigna angularis (azuki bean)	1	0	1
Rajma	5	5	10
Pea	6	2	8
Arhar	3	0	3
Gram	5	0	5
Cowpea	2	0	2
Urd bean	2	0	2
Lupine	2	4	6
Fiber Crops			
Cotton	5	0	5
Jute	5	1	6
Cash Crops			
Tobacco (*N. tabacum*)	1	4	5
Sugarcane	9	0	9
Vegetables			
Potato	1	0	1
Tomato	4	1	5
Onion	2	0	2
Green pepper	3	1	4
Lettuce	2	0	2
Currant	1	0	1
Others			
Forage crops	4	1	5
Fruit trees	13	0	13
Others	7	0	7
Total	225	112	337

developed at the Svalof station of the Swedish Seed Association. They represented a small but consistent increase in yield and oil content over the parent variety and it took 10-13 years to develop them. Further, there is some doubt that the improvements in these two varieties were due to irradiation since both the crops (mustard and rape) show a high degree of cross-pollination. Why should the cross-pollinated nature of the species raise doubts about the benefits arising from mutagenesis ?

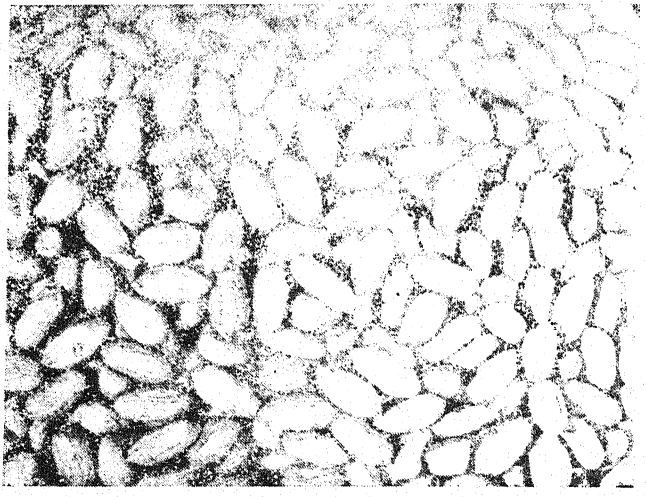


Fig. 23.8. Seeds of groundnut strain TG-19, a large pod selection from a mutant × mutant cross (Courtesy, Dr. C.R. Bhatia, BARC, Trombay).

Mutagenesis work in India started in 1930s on a small scale, but received considerable attention during late 1950s and 1960s. A number of crop varieties have been developed through mutagenesis, *e.g.*, NP 836 wheat, Indore 2 cotton, JRO 514 and JRO 412 jute (*C. olitorius*), Jagannath rice and Co 8152 and Co 8153 sugarcanes (*S. officinarum*) etc. (Table 23.2). NP 836 wheat is an awned mutant from the awnless wheat variety NP 799. Although the variety NP 836 has long been replaced by semidwarf wheat varieties, it illustrates the usefulness of mutagenesis in changing a single characteristic of an otherwise desirable variety. Jagannath rice is a gamma-ray induced semidwarf mutant from the tall cultivar T 141 ; Jagannath has an improved resistance to lodging, higher yielding ability and is more responsive to fertilizer application than the parent variety. Rice variety CRM 13-324 was developed through mutagenesis ; it has reduced height and days to maturity, and increased

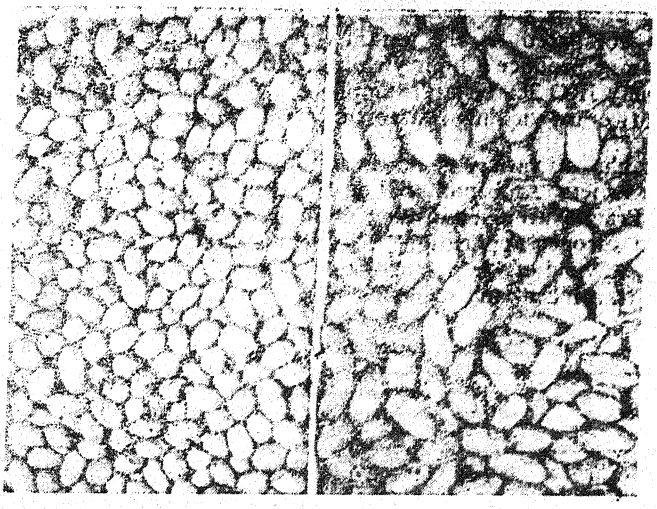


Fig. 23.9. Seeds of Spanish improved (on the left) variety of groundnut and of TG 19A (on the right) derived from the cross TG 1×TG 17, TG 1 is a large pod mutant of Spanish improved, while TG 17 was developed by intercrossing two mutant groundnut strains (Courtesy, Dr. C.R. Bhatia, BARC, Trombay).

Table 23.2. A list of some varieties developed in India through mutation breeding.

Crop	*Mutant variety*	*Parent variety*	*Mutagen*
Chickpea	BGM 408 (Pusa mutant 408)	G 130	
	BGM 413 (Pusa 413)	G 130	
	BGM 417 (Pusa 417)	BG 203	
	RSG 2	RS 10	
	RS 11	RS 10	
Pea	Hans		
Rabi cowpea	N 16	Pusa Phalguni	
	V 240	Pusa Phalguni	
Arhar	TV 1 (Trombay Vishakha-1)	T 21	γ-rays
	TAT 5	T 21	γ-rays
	TAT 70		γ-rays
Moong	TAT 7	S 8	γ-rays
	Pant Moong 2		
Urd	CO 4	CO 1	MMS (0.02%)
	TAU-1		γ-rays
Groundnut	TG 17	TG 1×TG 17 (TG 1 is a mutant)	
	TG 19A		
Jute	TKJ 40 (Mahadev)		γ-rays
Wheat	Sharbati Sonora	Sonora-64	γ-rays

yield as compared to the parent variety. Often two low yielding mutants may produce a high yielding progeny upon hybridization. Jute variety JRO 3690 is an example of this; it was produced by crossing two low yielding mutants of *C. olitorius*.

Prabhavati variety of rice is an induced mutant from the tall, scented variety Ambemohar Local which was treated with 0.2% EMS. Prabhavati is semidwarf, nonlodging and responsive to fertilizer and nitrogen application. It yields 35-40 Q/ha as compared to 25-30 Q/ha yield of the parent variety (Table 23.3). It has medium-slender, translucent and scented grains.

Table 23.3. Characteristics of Prabhavati Rice.

Variety	*Plant height (cm)*	*Days to maturity*	*1000-grain weight (g)*	*Straw yield (Q/ha)*	*Grain yield (Q/ha)*	*Lodging score**
Prabhavati (mutant)	75—80	115—120	23.8	45.6	32.3	0
Ambemohar (parent)	120—130	110—115	23.0	64.5	28.3	4

*Score 0—4; 0, no lodging; 4, very high degree of lodging.

Sugarcane variety Co 8152 is a gamma-ray induced mutant from Co 527. Co 8152 gives 40 per cent higher cane yield than the parent variety, but it has an apparently undesirable feature. The undesirable feature is that its lower leaves dry more quickly than in the parent variety and in other sugarcane varieties. This has limited its popularity with the farmers, although the mutant variety is higher yielding than the popular parent variety. This illustrates the undesirable side effects associated with desirable mutations. *The mutant variety Co 8152 has two chromosomes less than the parent variety Co 527. This is an example of a change in chromosome number producing a desirable mutant phenotype.* Co 8153 is a gamma-ray induced mutant from the sugarcane variety Co 775, which is highly susceptible to smut. The mutant variety is highly resistant to smut and is as productive as the parent variety. The chromosome number of this mutant variety is yet to be checked!

Three mutant cotton varieties have been released for commercial cultivation; two of these (MCU 7 and MCU 10) were developed through induced mutations, while one (DB-3-12) is a spontaneous mutant. MCU 7 was isolated from culture 1143 EE; it is early maturing (145 days), has medium staple length and is suitable for cultivation in rice fallow areas of Tamil Nadu. MCU 10 is a mutant from MCU 4 induced by 30 kR gamma-rays; it performs better than the parent variety (MUC 4) under rainfed conditions. DB-3-12 is a spontaneous mutant from the *G. herbaceum* variety Western 1; it has a better plant type and matures 20 days earlier than the parent variety.

Table 23.4. Proportion of the varieties developed through treatments with physical and chemical mutagens in sexually and asexually propagated crops.

Mutagenic treatment	*Sexually propagated crops*	*Asexually propagated crops*
Physical mutagens	89.2	98.8
Chemical mutagens	9.6	0.8
Physical and chemical mutagens combined	1.2	0.4

According to an estimate, about 94% of the mutant varieties developed till 1982 were developed following treatments with physical mutagens, about 5% through chemical mutagenesis and the remaining 1% through a combined treatment with both physical and chemical mutagens. Further, a much higher proportion of the mutant varieties owe their origins to physical mutagens in asexually propagated crops than in sexually reproducing ones (Table 23.4). This may not reflect a greater effectiveness of physical over chemical mutagens ; it may simply be related to the easier availability of the former resulting in its much more extensive application.

SUMMARY

Mutations are sudden heritable changes in the characteristics of organisms. Mutations may be chromosomal, cytoplasmic or gene mutations (point mutations). Mutations occur spontaneously at a low frequency, ca. 10^{-6} are usually recessive, deleterious in effect, random and recurrent in occurrence. Mutations can be induced at relatively higher frequencies by treatment with certain radiations (*physical mutagens*) or chemicals (*chemical mutagens*). Mutagenic action of X-rays was first discovered by Muller in 1927, and that of nitrogen mustards by Auerbach and Robson in 1946. Based on their effect on survival, mutations are classified into four groups : lethal, sublethal, subvital and vital. Mutation breeding utilizes vital mutations only.

In mutation breeding, gamma-rays and X-rays are the most commonly used physical mutagens, while EMS, EI and sodium azide are the most commonly used chemical mutagens. X-rays and gamma-rays act by causing ionisation directly in the DNA or in the medium which produces free radicals. *Free radicals* are highly reactive and produce H_2O_2 and organic peroxides which act on DNA. EMS and EI act primarily by chemical interaction with DNA, *e.g.*, alkylation of N at 9 position in guanine.

A mutation breeding programme should have a well defined and clear-cut objective, and the variety to be irradiated should be carefully chosen. Seeds, pollen grains, buds or cuttings may be used for mutagen treatment ; seeds are more commonly treated due to ease in handling. The dose of mutagen should be decided by experimentation ; a dose close to LD_{50} is generally preferred. Subsequent handling of the treated population in M_2 and later generations depends upon the mode of reproduction of the crop species, and on the oligogenic or polygenic nature of the mutations. About 9-10 generations (years) may be required in developing a mutant variety. *Recurrent mutation treatment* may be given, and whole plants may be irradiated at various stages of development at a gamma-garden. Gamma-gardens were established at Bose Institute Calcutta, IARI, New Delhi and BARC, Trombay.

Control on the direction of mutation (*directed mutagenesis*) is not feasible at present. Mutation breeding has been used to improve both oligogenic and

polygenic traits ; more than 300 varieties have been developed through mutagenesis in various countries. Mutation breeding is the only technique available for changing specific characters in clonal crops. Application of mutation breeding is limited by low frequency of desirable mutations, nonavailability of quick and efficient screening techniques, association of undesirable side effects, and recessiveness of most of the desirable mutations, particularly in polyploid and clonal crops.

QUESTIONS

1. Compare and differentiate between the following : (i) spontaneous and induced mutations, (ii) chromosomal and gene mutations, (iii) mutation and mutagen, (iv) UV and gamma radiations, (v) physical and chemical mutagens, (vi) oligogenic and polygenic mutations, (vii) cytoplasmic mutations and bud mutations, (viii) periclinal and sectorial chimeras. (ix) particulate and nonparticulate radiations, (x) ionising and nonionising radiations, and (xi) mutagenesis and mutation breeding.
2. Define the following : mutator genes, mutation breeding, ionisation, excitation, depurination, irradiation, M_2 generation, optimum mutagen dose and LD_{50}.
3. Define mutation. Briefly describe the characteristics of mutations and their effects on survival of organisms.
4. Define mutagen. List the different mutagens and briefly describe the properties and the mechanisms of action of either physical or chemical mutagens.
5. Write short notes on the following : (i) ionisation, (ii) free radicals, (iii) alkylating agents, (iv) gamma-garden, (v) recurrent irradiation, (vi) directed mutagenesis, (vii) UV rays, (viii) mutagenic DNA alterations, and (ix) chimeras.
6. Discuss the physical and chemical effects of radiations.
7. Outline a generalised procedure for mutation breeding with special reference to oligogenic characters.
8. Discuss the applications of mutation breeding in crop improvement.
9. Describe the achievements of mutation breeding and discuss the limitations of this approach of plant breeding.

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CHAPTER 24

Polyploidy in Plant Breeding

In discussions throughout this book, it has been assumed that the chromosome number of a species remains constant. The mitotic and meiotic divisions are very precise as a result of which the chromosome numbers of different species are highly stable. But a low frequency of irregularities do occur both during mitotic and meiotic divisions. These irregularities give rise to individuals with chromosome numbers different from the normal somatic chromosome number of the concerned species. Changes in chromosome number (some types) have contributed greatly to crop evolution, and (all the types) are of much use in plant breeding. In this chapter, we shall discuss in some detail the types of changes in chromosome number, their characterstics, production and applications in crop improvement.

TYPES OF CHANGES IN CHROMOSOME NUMBER

The *somatic chromosome number* of any species, whether diploid or polyploid, is designated as $2n$, and the chromosome number of gametes is denoted as n. An individual carrying the gametic chromosome number, n, is knwon as *haploid*. A *monoploid*, on the other hand, has the basic chromosome number, x. In a diploid species, $n=x$; one x constitutes a *genome* or *chromosome complement. The different chromosomes of a single genome are distinct from each other in morphology and/or gene content and homology ; members of a single genome do not show a tendency of pairing with each other.* Thus a diploid species has two, a triploid has 3 and a tetraploid has 4 genomes and so on.

The terminology of heteroploidy is summarised in Table 24.1. Individuals carrying chromosome numbers other than the diploid ($2x$ and not $2n$) number are known as *heteroploids*, and the situation is known as *heteroploidy*. The change in chromosome number may involve one or a few chromosomes of the genome ; this is known as *aneuploidy. The aneuploid changes are determined in relation to the somatic chromosome number ($2n$ and not $2x$) of the species in question.* Therefore, the terminology for aneuploid individuals arising from

Table 24.1. A summary of the terms used to describe heteroploidy (variation in chromosome number)

Term	*Type of change*	*Symbol**
Heteroploid	Change from $2x$	
A. Aneuploid	One or a few chromosomes extra or missing from $2n$	$2n \pm$ few
Nullisomic	One chromosome pair missing	$2n-2$
Monosomic	One chromosome missing	$2n-1$
Double monosomic	One chromosome from each of two different chromosome pairs missing	$2n-1-1$
Trisomic	One chromosome extra	$2n+1$
Double trisomic	One chromosome from each of two different chromosome pairs extra	$2n+1+1$
Tetrasomic	One chromosome pair extra	$2n+2$
B. Euploid	Number of genomes more than two	
Haploid	Gametic chromosome complement	n
1. Monoploid	One genome	x
2. Autopolyploid	Genomes identical with each other	
Autotriploid	Three genomes	$3x$
Autotetraploid	Four genomes	$4x$
Autopentaploid	Five genomes	$5x$
Autohexaploid	Six genomes	$6x$
Autooctaploid	Eight genomes	$8x$
3. Allopolyploid	Two or more distinct genomes (generally each genome has two copies)	
Allotetraploid	Two distinct genomes	$(2x_1+2x_2)$**
Allohexaploid	Three distinct genomes	$(2x_1+2x_2+2x_3)$**
Alloctaploid	Four distinct genomes	$(2x_1+2x_2+2x_3+2x_4)$**

*$2n$=Somatic chromosome number and n=gametic chromosome number of the species, whether diploid or polyploid.

x=The basic chromosome number or genomic number.

x_1, x_2, x_3, x_4=Distinct genomes from different species.

**In general, this condition occurs ; other situations may also occur.

diploid and polyploid species is the same. Heteroploidy that involves one or more complete genomes is known as *euploidy*. By definition, therefore, the chromosome number of euploids is an exact multiple of the basic chromosome number of the species, while that of aneuploids is not.

Aneuploid individuals from which one chromosome pair is missing ($2n-2$) are termed as *nullisomics*, while those lacking a single chromosome ($2n-1$) are known as *monosomics*. *A double monosomic* individual has two chromosomes missing, but the two chromosomes belong to two different chromosome pairs ($2n-1-1$). An individual having one extra chromosome ($2n+1$) is known as a *trisomic*, and that having two extra chromosomes each belonging to a different chromosome pair is *double trisomic* ($2n+1+1$). When an individual has an extra pair of chromosomes, it is known as *tetrasomic* ($2n+2$). The detailed terminology describing aneuploidy is very complex, and is beyond the scope of this book. The same is true about the various features of and the facts accumulated about

aneuploidy as well as euploidy. Here, I shall limit the discussion to those aspects that concern a breeder the most. *The breeder is generally concerned with monosomics and trisomics, and in some situations with nullisomics and tetrasomics.*

In *euploids*, the chromosome number is an exact multiple of the basic or genomic number. Euploidy is more commonly known as *polyploidy*. When all the genomes present in a polyploid species are identical, it is known as *autopolyploid* and the situation is termed as *autopolyploidy*. In the case of *allopolyploids*, two or more distinct genomes are present. Euploids may have 3 (*triploid*), 4 (*tetraploid*), 5 (*pentaploid*), 6 (*hexaploid*), 7 (*heptaploid*), 8 (*octaploid*) or more genomes making up their somatic chromosome number. In case of autopolyploidy, they are known as autotriploid, autotertaploid, autopentaploid, autohexaploid, autoheptaploid, autooctaploid and so on, while in the case of allopolyploidy they are termed as allotriploid, allotetraploid, allopentaploid, allohexaploid, alloheptaploid, allooctaploid etc. *Amphidiploid* is an allopolyploid that has two copies of each genome present in it, and as a consequence behaves as a diploid during meiosis. A *segmental allopolyploid* contains two or more genomes which are identical with each other, except for some minor differences

HISTORY OF HETEROPLOIDY

The first variation in chromosome number (heteroploidy) discovered in an experimental population was the gigas mutant in *Oenothera* described by Lutz in 1907. The gigas was an autotetraploid ($4n$). In 1910, Blakeslee discovered the globe mutant of *Datura stramonium* (dhatura) which was subsequently demonstrated by Belling (in 1920) to be a trisomic ; this was the first reported case of aneuploidy. The first autotetraploid was experimentally induced by Winkler in 1916 in *Solanum nigrum* (black nightshade) ; he decapitated the shoots, and some of the shoot buds arising from the callus thus produced were tetraploids. Winge in 1917 suggested that interspecific hybridization followed by chromosome doubling may be important in the evolution of new species. The first species synthesized experimentally was undoubtedly *Triticale* by Rimpau, in 1890, from a cross between *Titricum* and *Secale*. But its chromosome number was not studied till 1936. Therefore, the first synthetic species about which the full information was furnished was *Nicotiana digluta* obtained by spontaneous chromosome doubling of the F_1 hybrid from the interspecific cross *Nicotiana glutinosa* ($n=12$♀) × *N. tabacum* ($n=24$, ♂). *N. digluta* was synthesized by Clausen and Goodspeed in 1925. Chromosome doubling can be experimentally obtained in a relatively high frequency with colchicine treatment. The chromosome doubling action of colchicine was first described by Blakeslee and Nebel in 1937 independently of each other, but the effect of colchicine on mitosis was discovered in 1955.

ANEUPLOIDY

Of the various aneuploids, monosomics—in polyploid species, such as, tobacco, wheat and oats—and trisomics—in diploid species, *e.g.*, *Datura*, maize, bajra, tomato (*L. esculetum*), rye (*S. cereale*), pea (*P. sativum*), spinach (*S. oleracea*) etc.—are most commonly used in genetic studies. Nullisomics are viable in a few highly polyploid species only, *e.g.*, wheat and oats; they are not viable even in tobacco which is an allotetraploid. Therefore, we shall consider here trisomic and monosomic analyses. A trisomic is known as *primary trisomic* if the extra chromosome is the same as one of the haploid genome that is, it is not modified. In a *secondary trisomic*, the additional chromosome is an isochromosome. In an *isochromosome*, the two arms of the chromosome are identical. A *tertiary trisomic* has a translocated chromosome as the extra chromosome. For the present, we shall confine ourselves to primary trisomics.

Origin and Production

Spontaneous Aneuploids originate spontaneously at a low frequency. The earlier cases of aneuploidy were produced spontaneously in experimental populations. Meiotic irregularities lead to the formation of $n+1$ and $n-1$ gametes, *e.g.*, in *Datura* about 0.4 per cent of pollen is likely to be $n+1$.

Triploid Plants The best sources of aneuploids are triploid plants. Distribution of chromosomes at the first meiotic anaphase is irregular leading to the production of a whole range of aneuploids in the progeny.

Asynaptic and Desynaptic Plants. In asynaptic and desynaptic plants, few to all chromosomes are present as univalents at metaphase I of meiosis. In the progeny of such plants, a relatively high frequency of aneuploids occur.

Translocation Heterozygotes. A 3 : 1 disjunction of the ring or the chain of four chromosomes in a translocation heterozygote would produce one $n+1$ and one $n-1$ gamete. As a result, in the progeny of translocation heterozygotes a variable frequency of aneuploids are found.

Tetrasomic Plants. Tetrasomic ($2n+2$) plants would produce $n+1$ gametes in considerable frequencies. Therefore, when they are crossed with normal diploid or *disomic* ($2n$) plants, they produce a high frequency of trisomics. Where possible, tetrasomics may be maintained for the production of trisomics.

Morphological and Cytological Features

Aneuploids are generally weaker than diploids. Monosomics do not survive in diploid species, and nullisomics do not survive even in some polyploid species *e.g.*, in tobacco. All the 21 nullisomics

are available in the wheat variety Chinese Spring. A full series of monosomics, that is, monosomics for each chromosome of the haploid complement, is available in wheat (Chinese Spring), tobacco and oats. In diploid species, only trisomics survive ; full series of trisomics are available in maize, barley, peas, tomato, bajra, spinach and several other species. Trisomics vary considerably in vigour ; some may be comparable to the normal diploid, *e.g.*, trisome 5 (that is, trisomic for chromosome 5) in maize, while others are greatly reduced in vigour. Aneuploids generally exhibit distinct morphology and almost all the features of the plant are affected. Loss or gain of different chromosomes usually produces distinct morphological effects, but morphology is not always a reliable guide to the type of change at the chromosome level. Monosomics in wheat are often comparable to normal plants in morphology. Therefore, all the plants suspected to be aneuploid must be analysed cytologically for confirmation.

During meiosis, *nullisomics* generally show regular bivalent formation and chromosome distribution producing gametes with $n-1$ chromosomes. *Tetrasomics* are relatively less regular, they usually form one quadrivalent (involving four chromosomes) at metaphase I which often separates 2 : 2 at anaphase I. But sometimes the separation of the four chromosomes of the quadrivalent is not as regular ; therefore, all the gametes produced by a tetrasomic are not $n+1$. In *monosomics*, one chromosome does not have a pair and remains as a univalent at metaphase I. At anaphase I, the univalent may move to one of the two poles, may lag and be lost, or may divide (as in mitosis) into two chromatids which move to the opposite poles. Due to irregular behaviour of the univalent, monosomics produce an excess of $n-1$ gametes, *e.g.*, wheat produces 75% $n-1$ gametes. In *trisomics*, the extra chromosome often forms a trivalent with the other two homologous chromosomes, or may remain as a univalent. The univalent behaves irregularly ; hence the proportion of $n+1$ gametes is usually less than 50 per cent.

Transmission of the aneuploid condition is very poor through pollen grains, primarily due to a slower tube growth of aneuploid pollen. As a result, normal pollen outcompetes the aneuploid one. The transmission of aneuploidy through female gametes is considerably higher. There is evidence that *aneuploid seeds* are generally smaller, show somewhat reduced germination and seedlings may show lower viability ; all these lead to a reduced recovery of aneuploid progeny.

Applications in Crop Improvement

1. Aneuploids are useful in studies on the effects of loss or gain of an entire chromosome or a chromosome arm on the phenotype of the individual. Their study has clearly demonstrated that character expression is governed by a balance between a large number of genes present in the genome, that is, loss or gain of chromatin upsets the normal development.

2. Aneuploids are useful in locating a linkage group and a gene to a particular chromosome. By using a secondary or tertiary trisomic, the gene may be located to one of the two arms of a chromosome, or even to a part of the chromosome arm. The most important application of aneuploids is in locating genes on particular chromosomes ; this will be considered in some detail.

3. Study of aneuploids has shown the homoeology between A, B and D genomes of wheat (*T. aestivum*), since a chromosome of A genome would compensate for the loss of the corresponding chromosome from the genome B or D. For example, tetrasomic condition of 2B compensates for the nullisomic condition of 2A or 2D so that a tetra-2A nulli-2B or 2D appears normal.

4. Aneuploids are useful in identifying the chromosomes involved in translocation.

5. They are useful in the production of substitution lines. Chromosome substitution may be desirable for studying the effects of individual chromosomes of a variety or for the transfer of the genes carried by specific chromosomes of a variety into another one.

Aneuploid Analysis for Locating Genes on Particular Chromosomes

Genes may be located on particular chromosomes by nullisomic, monosomic or trisomic analysis. These analyses are briefly described below.

Nullisomic Analysis. Nullisomic analysis is restricted to a few polyploid species like wheat. Four approaches may be used for nullisomic analysis : (1) absence of expression of dominant character, (2) study of F_2 generation, (3) study of F_3 progenies, and (4) chromosome substitution.

Absence of Expression of a Dominant Character. When a dominant gene is located in a chromosome, the dominant character would not be expressed in the nullisomic for that chromosome. Genes for seed colour and awn inhibition in wheat have been located in this manner. Genes suppressing homoeologous pairing have been located on chromosome 5B because nulli-5B plants show a tendency for homoeologous pairing and multivalent formation instead of regular bivalent formation. But generally, genes present on homoeologous chromosomes or on other chromosomes mask the absence of a dominant gene in the nullisomic, *i.e.*, the nullisomic may still show the dominant phenotype. In such a case, study of the F_2 generation would be necessary.

Study of F_2 Generation. A strain carrying the dominant character is crossed to each of a complete set of nullisomics, *e.g.*, the 21 nullisomics in wheat. The progeny of such a cross would be all

monosomics (nullisomics producing $n-1$ and the normal producing n gametes giving rise to $2n-1$ zygotes on union) ; the unpaired chromosome is contributed by the normal strain. These monosomic plants are selfed and F_2 is raised separately for each monosomic. *F_2 family obtained from the nullisomic for the critical chromosome (carrying the dominant gene under study) would show very few plants with the recessive phenotype ; the plants with the recessive phenotype would all be nullisomics.* This is because on selfing monosomics produce normal, monosomic and nullisomic plants (Fig. 24.1). The normal and monosomic plants receive the critical chromosome (carrying the dominant gene) from the normal parent ; only the nullisomic progenies would not have this chromosome (Fig. 24.2). F_2 progenies from other nullisomics would show a 3 : 1 segregation for the character. Thus the nullisomic producing a F_2 family with a very small number of plants showing the recessive trait lacks the chromosome carrying the gene under study. This observation should be confirmed cytologically in that the plants showing the recessive character should all be nullisomics.

	♂	Pollen	
		n	$n-1$
		96%	4%
Egg cell	n 25%	$2n$ 24% (Normal)	$2n-1$ 1% (Monosomic)
	$n-1$ 75%	$2n-1$ 72% (Monosomic)	$2n-2$ 3% (Nullisomic)

Fig. 24.1. Frequency of different gametes and progeny produced by monosomics upon selfing. The frequencies are based upon data collected in wheat. Male transmission of the aneuploid ($n-1$) gamete ranges from 0—10 per cent with a mean of about 4 per cent, while about 75 per cent of the female gametes are $n-1$.

Study of F_3 Progenies. In some cases, it may be desirable to study F_3 progenies instead of F_2 plants. Normal disomic plants (plants having two homologues for each chromosome, *i.e.*, $2n$) from the F_2 generation are selfed to raise F_3 progenies. In the critical cross, all such F_3 families will be homozygous for the character in question and would show no segregation. But in other F_3 families, a normal 3 : 1 ratio will be obtained.

Chromosome Substitution. Individual chromosomes from a given variety may be transferred into a single variety to study the effects of individual chromosomes in a common genetic background. Alternatively, a chromosome carrying a desirable gene or a group of genes may be transferred to another variety lacking those genes. *The transfer of a pair of chromosomes from one strain into a different strain is known as chromosome substitution.*

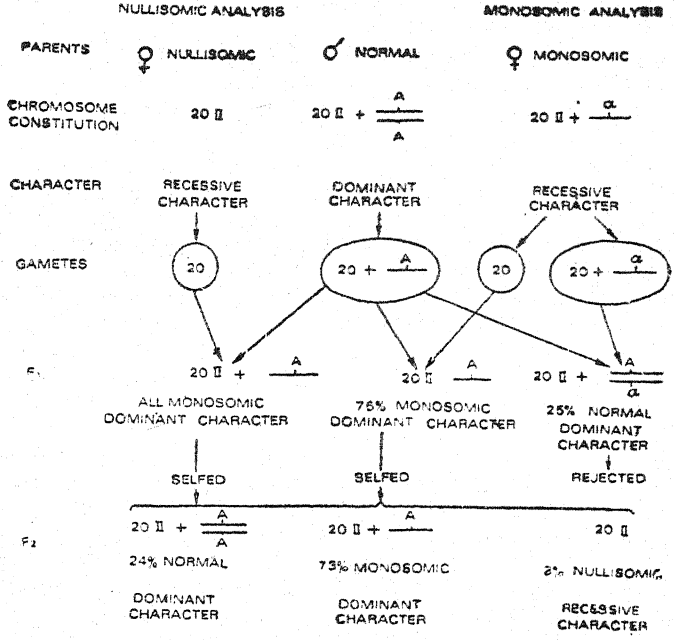


Fig. 24.2. Locating a dominant gene on a particular chromosome by studying the F_2 generation from a cross with the nullisomic/monosomic involving the chromosome carrying the gene in question. The results in F_2 from both monosomic and nullisomic analyses are the same. The only difference lies in F_1 ; nullisomics produce only monosomics, while monosomics produce monosomics as well as normal plants when crossed to a normal plant. Only monosomics are selfed to raise the F_2 ; they are identified by cytological analysis. The left hand part in the diagram depicts nullisomic analysis, while the right hand part shows monosomic analysis. F_2 generations from both the analyses are identical. The figure depicts aneuploid analysis in wheat.

Chromosome substitution is easily done using nullisomics of the variety into which the chromosome is to be transferred (the recurrent parent). More commonly, monosomics are used in place of nullisomics, but the operation in this case is relatively more complicated. The appropriate nullisomic is crossed with the strain ($2n$) from which a given chromosome is to be transferred. The resulting monosomic F_1 is backcrossed to the nullisomic (used as female), yielding 96 per cent monosomic and 4 per cent nullisomic progeny. The nullisomics are discarded. The monosomic progeny are repeatedly backcrossed to the nullisomic parent. After 6-7 backcrosses the monosomic plants are selfed ; they would produce about 24% normal disomic plants which would have one pair of chromosomes from the normal parent (in the original cross). It may be pointed out that the F_1 and the monosomic plants in the subsequent generation

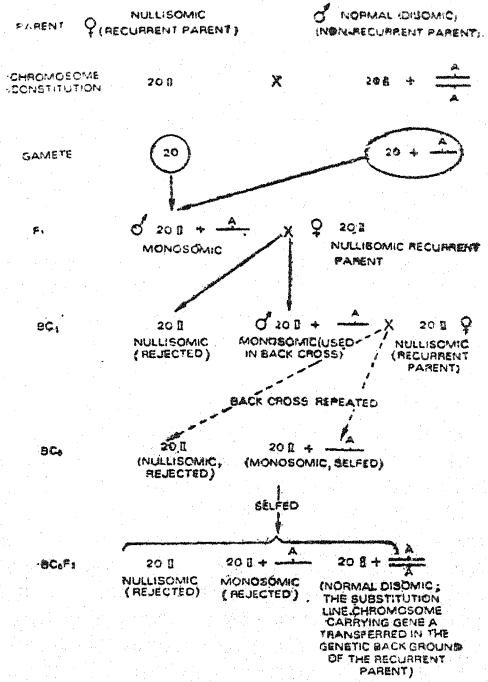
would receive an unpaired chromosome (for which the plants are monosomic) from the normal parent.

Monosomic Analysis. Monosomic analysis is useful in locating genes in particular chromosomes, and for chromosome substitution. Monosomic analysis was first used in *Nicotiana tabacum* by Clausen and was subsequently extended to other polyploid species.

Locating Genes in A Particular Chromosome. The complete set of monsomics (used as female) is crossed to a normal disomic strain carrying a dominant gene. In F_1, 25% of the plants would be $2n$; they are rejected. The remaining (75%) plants would be monosomics (identified by cytological analysis), which are selfed to produce the F_2 generation. In the F_2 derived from the monosomic for the critical chromosome (carrying the gene under study), all the plants would show the dominant character ; those showing the recessive trait would be nullisomics (Fig. 24.3). In tobacco, nullisomics do not survive, hence plants showing the recessive trait are absent in the critical family.

Chromosome Substitution. The appropriate monosomic is used as the recurrent parent in a backcross programme with the strain from which the chromosome is to be tranferred (the donor parent). The monosomic (used as female) is crossed to the donor parent. The F_1 has about 75% monosomic progeny, which are monosomic for the chromosome to be transferred. The monosomic F_1 plants are utilized in one of three ways.

1. *Monosomic F_1 plants are selfed* to produce $2n$ plants which have the critical chromosome in disomic condition, *i.e.*, having two homologues. Disomic plants are backcrossed to the appropriate monosomic of the recurrent parent. Monosomic progeny from the backcrosses are handled in the same manner as the monosomic F_1 progeny. Thus each backcross generation is followed by a selfed generation, and thus 6 backcrosses would require 12 generations. At the end of backcrossing, monosomic progeny are selfed and $2n$ progeny are isolated ; these would be disomic for the critical chromosome, and will constitute the substitution line.
2. The monosomic line of the recurrent parent may be *monosomic for a telocentric chromosome or an isochromosome*, which can be recognised from the normal chromosome under the microscope. If such a line is used as the recurrent parent, there is no need for selfing ; backcrosses follow each other in succession, and 6 backcrosses require only 6 generations. The monosomic recurrent parent is used as female, while those from F_1 and the backcross progeny are used as male.
3. Even when normal monosomic lines are used as recurrent parent, *backcrosses may be made in succession* without selfing the monosomic progeny (as in the case of item 2). One out of every 75 monosomics in such a case would be monosomic for the chromosome from the recurrent parent ; this chance in 6 backcrosses would be 1 out of 12.



F_1 backcrossed to the nullisomic recurrent parent.

Monosomic plants selected and backcrossed to the recurrent nullisomic parent.

Fig. 24.3. Chromosome substitution using a nullisomic for the critical chromosome as the recurrent parent. If a monosomic recurrent parent is used, monosomics from F_1 and each backcross generation are selfed to isolate normal disomic plants ; these are then backcrossed to the monosomic recurrent parent.

Trisomic Analysis. Trisomic analysis is applicable to diploid species where monosomic or nullisomic analyses are not possible. The strain carrying the gene to be located on a particular chromosome is crossed (as a male) to each of a complete set of trisomics (used as female). The F_1 would have trisomic and disomic plants ; disomics are discarded. Trisomic F_1 plants are either selfed or testcrossed to a recessive disomic strain. The resulting F_2 or the testcross progeny would show a 3 : 1 or 1 : 1 segregation, respectively, for the character if it is not governed by a gene present on the chromosome which is in the trisomic state. But the ratio would be significantly different from 3 : 1 or 1 : 1 if it is present on the trisomic chromosome.

Trisomic analysis has been done in maize, barley, tomato and some other crops.

Limitations of Aneuploid Analyses

1. It is necessary to produce and maintain a complete set of aneuploids. Production, identification and maintenance of aneuploids requires elaborate cytogenetic analysis, which is difficult, time consuming and requires considerable skill.
2. Maintenance of aueuploids is complicated by the phenomenon of univalent shift. *Univalent shift* denotes that some of the progeny of an aneuploid plant would become aneuploid for a different chromosome as compared to the parent plant. Univalent shift generally occurs in monosomic lines and is a result of univalent formation in a chromosome other than that for which they are monosomic. Therefore, cytological analysis and testing must form an integral part of the aneuploid programmes.
3. During aneuploid analysis and chromosome substitution, cytological analysis must be carried out for accuracy. This involves a considerable cytological work and makes aneuploid analysis a time consuming and tedious task.

AUTOPOLYPLOIDY

In autopolyploidy are included monoploidy, triploidy, tetraploidy and higher levels of ploidy. Production of monoploids and haploids is entirely different from that of other ploidy levels and shall be considered separately. Other cases of autopolyploidy are produced directly or indirectly through chromosome doubling which is briefly considered below. It may be pointed out that, strictly speaking, monoploidy and haploidy do not constitute polyploidy. But in view of their specialized application in crop improvement, they are included here for discussion.

Origin And Production of Doubled Chromosome Numbers

Cells/individuals having doubled chromosome numbers may originate in one of the following several ways : (1) spontaneous, (2) due to treatment with physical agents, (3) regeneration in vitro, (4) colchicine treatment, and (5) other chemical agents.

Spontaneous. Chromosome doubling occurs occasionally in somatic tissues and unreduced gametes are also produced in low frequencies. Production of unreduced gametes is promoted by certain genes, *e.g.*, genes causing complete asynapsis or desynapsis.

Production of Adventitious Buds. Decapitation in some plants leads to callus development at the cut end of stem. Such a callus has some polyploid cells, and some of the shoot buds regenerated from the callus may be polyploid. This is of common occurrence in Solanaceae where 6-36 per cent of adventitious buds are reported to be tetraploid. The frequency of polyploid buds may be increased

by the application of 1 per cent IAA at the cut end as it favours callus development.

Physical Agents. Heat or cold treatments, centrifugation and X-ray or gamma-ray irradiation may produce polyploids in low frequencies. Tetraploid branches were produced in *Datura* in response to cold treatment. Exposure of maize (*Z. mays*) plants or ears to a temperature of 38-45°C at the time of the first division of zygote produces 2-5 per cent tetraploid progeny. Heat treatment has been successfully used in barley (*H. vulgare*), wheat (*T. aestivum*), rye (*S. cereale*) and some other crop species.

Regeneration in Vitro. Polyploidy is a common feature of the cells during in vitro culture. Some of the plants regenerated from callus and suspension cultures may be polyploids. Plants of various ploidy have been regenerated from callus cultures of *Nicotiana*, *Datura*, rice (*O. sativa*) and several other species.

Colchicine Treatment. *Colchicine treatment is the most effective and the most widely used treatment for chromosome doubling.* It has been used with great success in a large number of crop species belonging to both dicot and monocot groups. Colchicine is a poisonous chemical isolated from seeds (0.2-0.8%) and bulbs (0.1-0.5%) of autumn crocus (*Colchicum autumnale*). It is readily soluble in alcohol, chloroform or cold water, but is relatively less soluble in hot water. Pure colchicine is $C_{22}H_{25}O_6N$. It blocks spindle formation and thus inhibits the movement of sister chromatids to the opposite poles. The resulting restitution nucleus includes all the chromatids ; as a result, the chromosome number of the cell is doubled. Since colchicine affects only dividing cells, it should be applied when the tissues are actively dividing. At any given time, only a small proportion of cells would be in division, hence repeated treatments should be given at brief intervals to double the chromosome number in a large number of cells of the shoot apex. The polyploid and diploid cells present in a shoot-tip compete with each other and diploid cells may often outcompete the polyploid ones. The degree of competition varies from species to species and even among varieties within species.

The method of colchicine application varies considerably. The different methods of treatment are briefly summarised below. Colchicine is usually applied as an aqueous solution. *Colchicine* is relatively unstable in an aqueous solution. Therefore, it is important to use freshly prepared aqueous solutions of colchicine.

1. *Seed Treatment* may be used for 1 to 10 days with concentrations from 0.001 to 1 per cent ; 0.2% is more common. Seeds are generally soaked in a shallow container to facilitate aeration.
2. *Seedlings* may be treated in a young stage. Germinating seeds may be inverted so that only young shoots are exposed to colchicine and roots are protected. Treatment duration may range from 3-24 hours.

3. *Growing shoot apices* are commonly treated with 0.1 to 1 per cent colchicine, which is applied by brush or with a dropper. The treatment is repeated once or twice daily for a few days. A small cotton wool piece may be placed at the shoot-tip which is daily soaked with colchicine solution. Alternatively, 0.5 to 1 per cent colchicine mixed in lanoline paste may be smeared on the shoot apex ; this treatment is repeated 2-3 times per week.

4. *In woody plants*, 1% colchicine is generally used for application on shoot buds. A small quantity of a wetting agent is added in the colchicine solution for a better wetting and penetration.

5. Special methods for the treatment *of cereals and grasses* have been developed. These methods aim at bringing the colchicine solution in contact with the shoot apical meristem.

Other Chemical Agents. Several other chemicals have polyploidizing effect. Notable among them are, acenaphthene, 8-hydroxyquinoline and nitrous oxide. These chemicals are much less effective than colchicine and are not commonly used.

Colchicine treatment may produce a low frequency of variable chromosome numbers (other than the doubled number) including haploidy, *e.g.*, in jowar (*S. bicolor*), tomato (*L. esculentum*) etc. Colchicine may also induce mutations without influencing the chromosome number. Mutants induced by colchicine generally breed true. These responses to colchicine may be considerably affected by the genotype of the variety being treated, light conditions, mineral nutrition etc. Available evidence suggests that colchicine may induce mutation alone, or mutation followed by chromosome reduction coupled with chromosome doubling ; the latter produces true-breeding mutations.

Morphological And Cytological Features of Autopolyploids

Morphological features of polyploids vary to some extent from one species to the other. Some general features are summarised below.

1. Polyploids have larger cell size than diploids. Guard cells of stomata are larger, and the number of stomata per unit area is lower in polyploids than in diploids.
2. Pollen grains of polyploids are generally larger than those of the corresponding diploids.
3. Polyploids are generally slower in growth and later in flowering.
4. Polyploids usually have larger and thicker leaves, and larger flowers and fruits which are usually less in number than in the diploids.
5. Polyploids generally show reduced fertility due to irregularities.

during meiosis and due to genotypic imbalance leading to physiological disturbances.

6. In many cases, autopolyploidy leads to an increase in general vigour and vegetative growth. But in some cases, polyploids are smaller and weaker.
7. Different species have different levels of optimum ploidy. For sugarbeet (*Beta vulgaris*), the optimum level is 3*x*, while for timothy grass (*Phleum pratense*) it is between 8-10*x*.
8. Autopolyploids generally have a lower dry matter content than diploids. As a result, the increased size of many polyploids may not represent an increased dry matter production, *e.g.* tetraploid turnip (*Brassica rapa*) and cabbage (*B. oleracea*).

The cytological features vary with the level of ploidy. *In monoploids*, the chromosomes do not pair and their distribution at anaphase I is random leading to an almost complete sterility. Some functional gametes with *n* chromosomes may be produced which may give rise to 2*n* progeny. In *triploids*, a variable number of trivalents, bivalents and univalents are produced. At anaphase I, chromosome separation in trivalents and distribution of univalents is irregular producing a range of aneuploid gametes. As a result, they produce various types of aneuploid progeny, *e.g.*, trisomics, double trisomics etc. Autotriploids are generally highly sterile, *e.g.*, watermelons, banana (*Musa paradisiaca*) etc., but in some cases they are highly fertile, *e.g.*, spinach (*Spinacea oleracea*). *Autotetraploids* show quadrivalents, trivalents, bivalents and univalents at metaphase I. They show variable fertility in different species, but the fertility is generally lower than that in diploids. In the progeny of tetraploids, the majority of plants are tetraploids, but some plants may show variation in chromosome number. There is considerable evidence that fertility of autotetraploids can be improved through selection. This has been achieved in many cases, *e.g.*, maize (*Z. mays*). toria (*B. campestirs* var. *toria*), rye (*S. cereale*), bajra (*P. americanum*), rice (*O. sativa*) etc. In the case of tetraploid Petkus rye. the fertility increased from 60% to 75% after 6 years of selection.

Segregation in Autotetraploids

Segregation in autotetraploids is much more complex than in diploids. This topic has been considered by Allard (1960) in some detail, and in this text a brief summary will be given. In an autotetraploid, 4 chromosomes are homologous to each other, hence each gene has 4 copies. A *simplex* individual has one dominant and 3 recessive alleles (*Aaaa*), a *duplex* has 2 dominant and 2 recessive alleles (*AAaa*), a *triplex* has 3 dominant and 1 recessive allele (*AAAa*), a *quadruplex* has all dominant alleles (*AAAA*), while a *nulliplex* has none (*aaaa*).

Let us consider segregation in a simplex (*Aaaa*). When the gene *A* is located close to centromere, crossing over between the

centromere and the gene would not take place. In this case, both the sister chromatids of each chromosome are attached to the same centromere and would move to the same pole at anaphase I. At anaphase II, the sister chromatids carrying the dominant allele would move to the opposite poles. Therefore, the two dominant alleles do not reach the same gamete, *i.e.*, *AA* gamete is not produced. Such a segregation is known as *random chromosome segregation.* Complete chromosome segregation (complete absence of crossing over between centromere and the gene in question) in a simplex would produce two types of gametes *Aa* and *aa*, in the ratio 1 : 1. Self-pollination of a simplex would produce three genotypes *AAaa*, *Aaaa*, and *aaaa*, in the ratio 1 : 2 : 1, giving the phenotypic ratio of 3 : 1 if a single dominant allele *A* is able to produce the dominant phenotype. It is assumed that at metaphase I only quadrivalents are formed and that separation of chromosomes is random.

When crossing over occurs between the centromere and the gene, the two sister chromatids carrying the dominant allele become attached to two different chromosomes (or centromeres). The two chromosomes involved in the crossing over (each now carrying a single dominant allele) may move to the same pole at anaphase I. At anaphase II, the two chromatids carrying the *A* allele may move to the same pole, and thus end up in the same gamete producing an *AA* gamete. This type of segregation is termed as *random chromatid segregation.* Random chromatid segregation produces 3 types of gametes, *AA*, *Aa* and *aa*, in the ratio 1 : 12 : 15. It is assumed that all the chromosomes are associated as quadrivalents, and that 50 per cent crossing over occurs between the gene and the centromere. Selfing of a simplex in such a case is expected to produce the following progeny.

Quadruplex	*AAAA*	1
Triplex	*AAAa*	24
Duplex	*AAaa*	174
Simplex	*Aaaa*	360
Nulliplex	*aaaa*	225
		784

The two assumptions in the above estimation are related to the regularity of quadrivalent formation and of complete or no linkage between centromere and the gene in question. These assumptions are rarely, if ever, fulfilled. Generally, quadrivalents are not regularly produced , a variable number of bivalents, univalents and trivalents are also formed. Further, genes and centromeres are generally partially linked. Therefore, the actual gametic and zygotic frequencies are intermediate between the two extremes set by random chromosome and random chromatid segregations, since one, the other or both of the assumptions are bound to be incompletely satisfied.

Role of Autopolyploidy in Evolution

Autopolyploidy has contributed to a limited extent in evolution of plant species. Some of our present day crop species are autopolyploids, *e.g.*, potato (4*x*), peanut (4*x*), coffee (4*x*), alfalfa (4*x*), banana (3*x*) and sweetpotato (6*x*). Autotetraploids appear to have been more successful as crops than other forms of autopolyploidy (Table 24.2). In addition, many forage grasses and several ornamentals are most likely autopolyploids.

Table 24.2. Autopolyploid crop species.

Common name	*Scientific name*	*Somatic chromosome number (2n) of the cultivated form*	*Somatic chromosome number of related wild species*
Potato	*Solanum tuberosum*	48 (4*x*)	24 (2*x*) form of *S. tuberosum*
Coffee	*Coffea arabica*	44 (4*x*)	22, 66, 68
Alfalfa	*Medicago sativa*	32 (4*x*)	14, 16, 32
Peanut	*Arachis hypogaea*	40 (4*x*)	
Banana	*Musa sapientum* (*M. paradisiaca*)	33 (3*x*)	22
Sweetpotato	*Ipomoea batatas*	90 (6*x*)	

Application of Autopolyploidy in Crop Improvement

Autopolyploidy has found some valuable applications in crop improvement. These are briefly summarised below.

Monoploids and Haploids. Monoploids are weaker than diploids and are of little agricultural value directly. But they are of great interest because they offer certain unique opportunities in crop improvement. (1) They are used for developing homozygous diploid lines, following chromosome doubling in two years. This greatly reduces the time and labour required for the isolation of inbreds and purelines. (2) They may be useful in the isolation of mutants because the mutant allele (even if it is recessive) expresses itself in M_1 due to a single dose of the gene in somatic tissues. Chromosome number of mutants may be doubled to produce homozygous mutant lines in a single generation. (3) Since desirable gametes are more frequent (p) than desirable zygotes (p^2), selection based on haploids or haploid-derived diploids may be expected to be more efficient than that based on diploid (zygote-derived) plants. There is some evidence that this may be so. And (4) in autotetraploids like potato, breeding is relatively much easier at the haploid (2*x*) level than at the tetraploid level (4*x*). For comparison, consider segregation in an autotetraploid and in a diploid. There is an increasing tendency to breed potato varieties at the haploid level and then double their chromosome number to obtain tetraploid varieties.

Monoploids and haploids occur spontaneously (*e.g.*, in maize, Chapter 19) in low frequencies, may be induced from pollen grains through callus formation or embryoid production (Chapter 26) and

by chromosome elimination in certain interspecific crosses, *e.g.*, *Hordeum bulbosum* × *H. vulgare* (Chapter 25). In the first method, the recovery of haploids is generally very low (1 in 1,000 plants or lower). But the latter two methods produce a relatively high frequency of haploids in case of those species for which appropriate techniques are available. It may be pointed out that the latter two methods are not applicable to many crop species as yet.

Triploids. Triploids are produced by hybridization between tetraploid and diploid strains. They are generally highly sterile, except in a few cases. This feature is useful in the production of seedless watermelons. In certain species, they may be more vigorous than the normal diploids, *e.g.*, in sugarbeets. These two examples are described in some detail.

Seedless watermelons are grown commercially in Japan. They are produced by crossing tetraploid ($4x$, used as female) and diploid ($2x$, used as male) lines, since the reciprocal cross ($2x \times 4x$) is not successful. The triploid plants do not produce true seeds ; almost all the seeds are small, white rudimentary structures like cucumber (*Cucumis sativus*) seeds. But a few normal sized seeds may occur, which are generally empty. For good fruit setting, pollination is essential. For this purpose, diploid lines are planted in the ratio 1 diploid : 5 triploid plants. There are several problems, *viz.*, genetic instability of $4x$ lines, irregular fruit shape, a tendency towards hollowness of fruits, production of empty seeds and the labour involved in triploid seed production (by hand-pollination).

Triploid sugarbeets (*B. vulgaris*) produce larger roots and more sugar per unit area than diploids, while tetraploids produce smaller roots and lower yields than diploids. Apparently, $3x$ is the optimum level of ploidy in sugarbeets. Triploid sugarbeet varieties have been grown commercially in Europe and Japan, but their popularity is declining rapidly. The triploid varieties are mixtures of triploid, diploid and other ploidy level plants. Seed production of triploid sugarbeet is difficult because the beet flower is small. Triploid seed may be produced in one of two ways : (1) using $4x$ plants as female and $2x$ as male or (2) using $4x$ as male and $2x$ as female. The first combination gives lower seed yield but a higher proportion of triploids, while the second gives a higher seed yield but a lower proportion of triploids. Commercial triploid sugarbeet seed is produced by interplanting $4x$ and $2x$ lines in the ratio of 3 : 1, and seed from both $4x$ and $2x$ plants is harvested. This seed consists of about 75% triploid ($3x$) seeds. Triploid sugarbeet may give 10-15 per cent higher yields than diploids.

Tetraploids. Autotetraploids have been produced in a large number of crop species and have been extensively studied in several cases. Tetraploids may be useful in one of the following ways : useful in breeding, improved quality, overcoming self-incompatibility, making distant crosses and used directly as varieties.

In banana (*M. sapientum*), autotetraploids are inferior to triploids in that they have weaker leaves and increased fertility. But

they offer the only available chance of adding disease resistance to commercially successful varieties. In banana, autoteraploids are produced by chance fertilization of an unreduced triploid egg (AAA) by a haploid pollen from a disease resistant diploid parent. A large number of such tetraploids have been produced, but they have not yet gained any commercial success. This is an unusual case where the *autotetraploidy is the only practical approach to breeding* an otherwise successful crop species.

Some autotetraploids may be *superior in some quality characters* to their respective diploids, *e.g.*, tetraploid maize has 43% more carotenoid pigment and vitamin A activity than the diploid. Some teraploids may be more hardy than diploids. However, it is impossible to predict the performance of tetraploids, and a superior diploid may not necessarily produce a superior tetraploid. The tetraploids may be superior, inferior or comparable to the corresponding diploids in quality and hardiness ; the actual response has to be determined experimentally.

Autotetraploidy is able to *overcome self-incompatibility* in certain cases, *e.g.*, some genotypes of tobacco (*Nicotiana* sp.) and white clover (*Trifolium repens*), *Petunia* etc. Certain distant crosses are not successful at the diploid level, but are relatively successful at the autotetraploid level, *e.g.*, 4*x Brassica oleracea* × *B. chinensis* is successful, but when *B. oleracea* is diploid it is unsuccessful. Similarly, autotetraploids of certain *Solanum* species produce hybrids with *S. tuberosum* while diploids do not.

Autotetraploids are larger in size and are more vigorous than diploids. Antotetraploid varieties of forage crops have been considerably successful. The most successful examples are, tetraploid red clover (*Trifolium pratense*) and ryegrass (*Lolium perenne*). Other examples are : tetraploids of alsike clover (*Trifolium hybridum*, variety Tetra) and berseem (*Trifolium alexandrium*, variety Pusa Giant Berseem). Autotetraploid red clover and ryegrass are more vigorous, digestible and palatable, and have greater resistance to nematodes as compared to the diploids. Autotetraploid turnips (*B. rapa*) and cabbages (*B. oleracea*) are larger in size, but they also have more water content than the diploids ; thus they are not commercially attractive. Many ornamentals are autotetraploids. In cases of ornamentals, increased flower size, and longer flowering duration of the tetraploids are desirable. Pusa Giant Berseem is the first polyploid variety released for general cultivation in India. It yields 20-30 per cent more green fodder than the diploid berseem varieties.

In case of crops where seed is the commercial product, autotetraploidy has been much less successful. The chief difficulty is the high sterility and genetic instability of autotetraploids. Fertility can be improved through breeding and selection, but the progress is slow. Autotetraploids have been explored in several crop species but the most successful case is that of rye (*S. cereale*) where tetraploid varieties have been released for cultivation (*e.g.*, Double Steel, Tetra Petkus). Other extensive programmes on autotetraploidy are on crops

like barley (*H. vulgare*) and jowar (*S. bicolor*) where larger grains, increased protein content and higher yields are the objective. After many years of extensive breeding, some success in achieving these goals has been realised. Some generalisations may be made about autopolyploidy.

1. Autopolyploidy is more likely to succeed in species with lower chromosome numbers than in those with higher chromosome numbers.
2. Cross-pollinating species are generally more responsive than self-pollinating species.
3. Crops grown for vegetative parts are more likely to succeed as polyploids than those grown for seeds.

Limitations of Autopolyploidy

1. The larger size of autopolyploids is generally accompanied with a higher water content. As a result, autopolyploids of the crop species grown for vegetative parts do not always produce more dry matter than the respective diploids. For example, tetraploid turnip (*B. rapa*) and cabbage (*B. oleracea*) outyield the diploids in fresh weight, but are comparable, or even inferior, to them in terms of dry matter production.
2. In crop species grown for seed, autopolyploids show high sterility accompanied with poor seed set. Consequently, the larger seed size of autotetraploids does not generally lead to an increased seed yield per unit area.
3. Fertility in autotetraploids can be increased by hybridization and selection at the tetraploid level. But due to the complex segregation in autotetraploids, progress under selection is slow. It would take many years to raise the fertility to acceptable levels.
4. Monoploids and triploids cannot be maintained, except through clonal propagation. The progeny of triploids and tetraploids are variable in chromosome number since they produce aneuploid gametes as well. Triploids have to be regularly produced by crossing $4n \times 2n$ plants. Maintenance of tetraploids is somewhat less difficult. Thus genetic instability of autotriploids and autotetraploids makes their maintenance difficult, and commercial seed production presents many problems.
5. The hope that polyploidy would help to create new agricultural types at will was entirely misplaced. New polyploids (raw polyploids) are always characterised by a few or more undesirable features, *e.g*, poor strength of stem in grapes (*Vitis vinifera*), irregular fruit size in watermelons etc. Thus new polyploids can rarely be used directly in crop production. A considerable improvement through hybridization and selection is essential to remove these defects.

6. Effects of autopolyploidy cannot be predicted. Therefore, one has to try and find out if it would be of some use in a given crop species.

ALLOPOLYPLOIDY

Allopolyploids have genomes from two or more species. Several of our crop plants are allopolyploids. Production of allopolyploids has attracted considerable attention ; the aim almost always was the creation of new species. Some success has been obtained as is evident from the emergence of *Triticale* as a new crop species in some areas, and the promise showa by some other allopolyploids, *e.g.*, *Raphanobrassica* and some allopolyploids of forage grasses.

Origin And Production of Allopolyploids

The present-day allopolyploids were most likely produced by chromosome doubling in F_1 hybrids between two distinct species (distant hybrids) belonging to the same genus or to different genera. Chromosome doubling might have occurred in somatic tissues due to an *irregular mitotic cell division* leading to the formation of allopolyploid sectors either in the apical meristem or in the axillary buds ; the latter would produce allopolyploid branches. Sexual progeny from such branches would be allopolyploids. Alternatively, *irregular meiosis* may lead to the production of unreduced gametes which may unite to produce allopolyploid progeny. A complete failure of pairing may be followed by the inclusion of all the chromosomes in a single nucleus at telophase I. This would lead to the production of two unreduced gametes after telophase II.

In experimentally produced distant hybrids, both the processes generating allopolyploidy have been observed. In many distant hybrids that were highly sterile, occasional branches were highly fertile and had twice as may chromosomes as those present in the F_1 hybrid, *e.g.*, F_1 from the cross *Primula verticillata* × *P. floribunda* produced a fertile branch with twice the chromosome number. The progeny obtained from this fertile branch were allotetraploid and were named as *P. kewensis*. In many other distant hybrids, occasional unreduced gametes are produced which unite to give rise to allopolyploid progeny. The allotetraploid *Raphanobrassica* was obtained in this manner ; the F_1 from the cross *B. oleracea* (cabbage) × *Raphanus sativus* (radish) was almost completely sterile, but produced a few seeds that gave rise to the allotetraploid *Rophanobrassica*.

Experimental production of allopolyploids is achieved by doubling the chromosome number of distant hybrids with the help of colchicine or some other agent. The allopolyploids produced by man are often termed as *synthetic allopolyploids* because they are produced (or so to say synthesized) by man from two distinct species of plants. Thus the production of an allopolyploid involves two steps : (1) production of F_1 distant hybrid, and (2) chromosome

doubling (Fig. 24.4). Many distant crosses not possible previously have been successfully made with the help of *in vitro* techniques. Chromosome doubling does not appear to be a serious problem in most of the cases since most of the plant species respond to colchicine. Some species may respond poorly to colchicine ; in such cases other agents available for chromosome doubling may be useful.

Morphological And Cytological Features of Allopolyploids

Allopolyploids generally combine the morphological and physiological characteristics of the parent species. But it is very difficult to

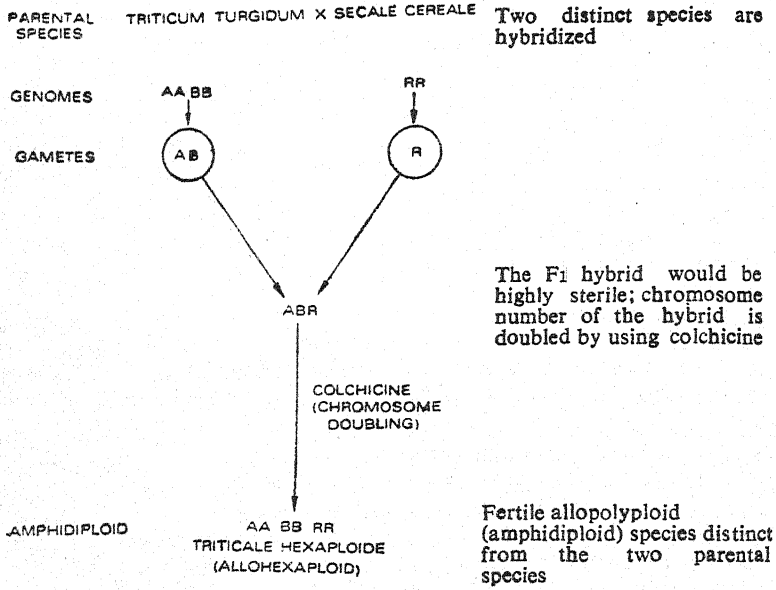


Fig. 24.4. Experimental production (synthesis) of an allopolyploid. The allopolyploid synthesised here is an allohexaploid, the hexapolyploid *Triticale* (*Triticale haxaploide*).

predict the precise combination of characters that would appear in the allopolyploid species. For example, the aim in producing *Raphanobrassica* was to synthesize a crop species that would combine the root of radish (*R. sativus*) and the leaves of cabbage (*B. oleracea*). *Raphanobrassica* did combine the characteristic root and shoot systems of the two parental species, but in the opposite direction, that is, it had leaves like radish and roots like cabbage ! On the other hand, *Triticale* has combined the favourable features of the two parental species, *i.e.*, the hardiness of rye (*S. cereale*) and the yielding ability of wheat (*Triticum* sp.). In general, allopoly-

ploids are more vigorous than diploids, but this also is not true in all the cases. The natural distribution of allopolyploids is usually different from those of the ancestral diploid species. This would indicate that allopolyploids differ in their adaptability from their parental species. Often they are hardier than the parental species, but rarely they are able to displace the parental species from the areas of their adaptation. Thus evolution of allopolyploidy in nature has been favoured by the availability of new ecological areas for the establishment of allopolyploids.

Another feature of allopolyploids is that many of them are apomictic. Apomictic species are common in grasses, *e.g.*, *Poa*. Apomicts are known in many other groups of plants, *e.g.*, *Taraxacum*, *Parthenium*, *Rubus*, *Fritillaria*, *Tulipa*, *Solanum*, etc. Apomixis arises in response to the near complete sterility of interspecific hybrids. Such sterile plants are able to reproduce asexually through apomixis.

The characteristics of allopolyploids would result from an interaction between the genetic systems of the two parental species that have evolved independently of each other. The nature of the interaction between the two systems may vary greatly from one allopolyploid to the other, which probably is partly responsible for the difficulty in predicting the effects of allopolyploidy. Another factor that is involved in allopolyploids is that two genomes of a species are transferred into the cytoplasm of another species. The cytoplasm-nucleus interactions may be of some importance in determining the characteristics of allopolyploids.

Chromosomes of distinct species may be expected to have differentiated to various degrees ; chromosomes of closely related species would be more similar to each other than those of unrelated species. Thus chromosomes of related species would be partially similar to each other (as against exactly identical homologous chromosomes) and are known as *homoeologous chromosomes*. Successful hybridization between two species shows some degree of relationship between them ; hence interspecific hybrids would have two or more homoeologous (not homologous) chromosomes. These homoeologous chromosomes may show various degrees of chromosome pairing depending upon the degree of homoeology. After chromosome doubling, the allopolyploid would have two homologous chromosome for each chromosome present in the F_1 hybrid, which is the same situation as in the diploid species. Such an allopolyploid, therefore, is commonly known as *amphidiploid* or *amphiploid*.

When the two genomes in an amphidiploid are divergent, that is, they have little homoeology, chromosome pairing would be highly regular and only bivalents would be produced at metaphase I. Such amphidiploids would be highly fertile, would be stable both genetically and cytologically and would show only bivalents at metaphase I. But if the two genomes are sufficiently homoeologous to show some chromosome pairing in the F_1 hybrid, the amphidiploid would show a variable number of quadrivalents. Such

amphidiploids would be partially fertile, unstable genetically and cytologically, and would show some quadrivalents at metaphase I. However, these generalisations are not always true, and many exceptions are known. Sterility is a common feature of amphidiploids and is most likely produced by physiological disturbances resulting from genetic imbalance. Irregular chromosome pairing, if present, is also a contributing factor.

Fertility of allopolyploids can be improved through hybridization and selection. Selection not only improves chromosome behaviour, but it also improves the gene combinations through recombination. Thus there is an increase in bivalent formation as a result of selection so that established polyploids become identical to diploids in terms of chromosome beaviour, *i.e.*, they become *diploidized*. The process due to which allopolyploids shows diploid-like behaviour, *i.e.*, only bivalent formation, is known as *diploidization*. Recombination leads to the development of gene combinations that improve physiological and cytological functions in allopolyploids. Natural allopolyploids have evolved highly efficient genetic systems that ensure regular bivalent formation by preventing pairing between homoeologous chromosomes. An excellent example is provided by the homoeologous pairing suppressing action of chromosome 5B in wheat. Wheat (*T. aestivum*) lines lacking chromosome 5B, *e.g.*, nulli-5B, show homoeologous pairing and multivalent formation. Similar genetic systems that suppress homoeologous pairing occur in the other established allopolyploids like oats (*A. sativa*), tobacco (*N. tabacum*) etc.

Role of Allopolyploidy in Evolution

Allopolyploids have been more successful as crop species than autopolyploids. Many of our present-day crop species are allopolyploids (Table 24.3). Allopolyploidy has contributed to a great extent in the evolution of plants. This is evident from the widespread occurrence of allopolyploidy in various genera of plants and from the great success allopolyploids have enjoyed in natural populations. It is estimated that about one-third of the Angiosperms are polyploids, and by far the vast majority of them are allopolyploids. In 1917, Winge postulated that allopolyploidy was important in evolution ; subsequent studies have clearly shown the validity of his suggestion.

It has been possible to trace back the evolutionary history of many allopolyploid crop species, and the diploid parental species have been identified with some degree of certainty. The identification of parental diploid species is primarily based on *pairing between the chromosomes of the diploid and the allopolyploid species.* When the chromosomes of a diploid species pair with some of those of the allopolyploid species, homology between the chromosomes of the two species is apparent. This homology suggests that the diploid species may be one of the parental species of the allopolyploid. But in many cases, considerable chromosome differentiation may have

Table 24.3. Some genera which contain allopolyploid species, and one or more crop species. The crop species themselves may be allopolyploid or diploid. Genera like *Triticum, Brassica* and *Gossypium* have both diploid and allopolyploid crop species.

Scientific name	*Common name*	*Gametic chromosome number* (n)	*Cultivated/Wild*
Avena strigosa	Sand oats	7	W
A. barbata	Slender wild oats	14	W
A. sativa	Cultivated oats	21	C
A. byzantina	Cultivated red oats	21	C
Brassica nigra	Black sarsson	8 (B)*	C
B. oleracea	Cabbage	9 (C)	C
B. campestris	Rape, turnip rape	10 (A)	C
B. carinata	Abyssinian cabbage	17	W
B. juncea	Rai, Indian mustard	18	C
B. napus	Rape	19	C
Gossypium arboreum	Asiatic (desi) cotton	13 (A_2)	C
G. herbaceum	Asiatic cotton	13 (A_1)	W
G. thurberi	Wild American cotton	13 (D_1)	W
G. barbadense	Sea island (Egyptian) cotton	26 (A_2D_2)	C
G. hirsutum	American upland cotton	26 (A_1D_1)	C
Hordeum vulgare	Cultivated barley	7	C
H. jubatum	Squirrel-trail barley	14	W
H. nodosum	Foxtail barley	21	W
Medicago hispida	California burclover	7	C
M. lupilina	Black medic	8, 16	C
M. falcata	Yellow alfalfa	8, 16	C
Nicotiana sylvestris	Wild tobacco	12	W
N. tomentosa	Wild tobacco	12	W
N. tabacum	Cultivated tobacco	24	C
N. rustica	Cultivated tobacco	24	C
N. bigelovii	Wild tobacco	24	W
N. debneyi	Wild tobacco	24	W
Prunus americana	American plum	8	C
P. avium	Sweet cherry	8	C
P. persica	Peach	8	C
P. cerasus	Sour cherry	16	C
P. domestica	European plum	16	C
Saccharum officinarum	Noble canes	40	C
S. barberi	Indian canes	41, 45, 46, 58, 62	C
S. sinense	Indian canes	58, 59	C
S. spontaneum	Kans (wild canes)	20-64	W
S. robustum	Wild cane	30-74	W
Sorghum versicolor	Wild sorghum	5	W
S. bicolor	Jowar	10	C
S. halepense	Johnson grass	20	C
Trifolium pratense	Red clover	7	C
T. alexandrium	Berseem clover	8	C
T. repens	White clover	16	C
T. medium	Zigzag clover	40, 48, 42, 49	C
Triticum boeticum	Wild einkorn	7 (A)	W
T. monococcum	Einkorn wheat	7 (A)	C
T. dicoccoides	Wild emmer	14 (AB)	W
T. dicoccum	Emmer wheat	14 (AB)	C
T. turgidum	Solid stem wheat	14 (AB)	C
T. carthlicum	Persian wheat	14 (AB)	C

T. polonicum	Polish wheat	14 (AB)	C
T. durum	Durum wheat	14 (AB)	C
T. timopheevii		14 (AG)	W
T. americanum		14 (AG)	W
T. spelta	Spelt wheat	21 (ABD)	C
T. aestivum	Common bread wheat	21 (ABD)	C
T. macha		21 (ABD)	W
T. vavilovi		21 (ABD)	W
T. compactum	Club wheat	21 (ABD)	C
T. sphaerococcum		21 (ABD)	W

*Letters within parentheses denote the symbols used for genomes present in the species.
C = Cultivated ; W = Wild.

taken place both in the diploid and the polyploid species, and pairing between the chromosomes of these species may be greatly reduced. Additional evidence on the parental diploid species is obtained by *synthesizing the naturally occurring allopolyploid from the parental diploid species*. The synthetic allopolyploid does often resemble in many ways the natural allopolyploid species. This has been clearly demonstrated in *Brassica* ; for example, *B. napus* (turnip rape) synthesized from *B. oleracea* (cabbage) and *B. campestris* (rape) resembles *B. napus* very closely indeed. But the resemblance between the synthetic and the natural allopolyploid often may not be as great, and sometimes it may be very poor. Confirmatory evidence on parental species is obtained by using *biochemical techniques*, such as, electrophoretic patterns of proteins and enzymes, and from chromosome banding patterns. We shall briefly consider the possible evolutionary history of some important allopolyploid crop species, *viz.*, wheat, tobacco, cotton and *Brassica*.

Evolution of Bread Wheat (*Triticum aestivum*). Evolutionary history of wheat has been the most extensively investigated, and is perhaps the least understood. Identity of the diploid species contributing the three genomes (A, B and D genomes) of *T. aestivum* has been investigated by many workers, more notably by Sears, Kihara and others. It is generally accepted that the A genome present in diploid wheats is the same or very similar to those present in tetraploid and hexaploid wheats. Further, the B genome of tetraploid emmer wheats is similar to that found in hexaploid wheats. This is evident from chromosome pairing in crosses among diploid, tetraploid and hexaploid wheats. Hybrids between diploid and tetraploid wheats show about 7 II and 7 I, while those between tetraploid and hexaploid wheats show about 14 II and 7 I. The above is generally widely accepted. But the diploid species contributing the A, B and D genomes are not agreed upon. It has been proposed that the source of A genome is *T. monococcum*, of B genome is *Aegilops speltoides* and of D genome is *Aegilops squarrosa*. This is summarised in Fig. 24.5 and in Table 24.4.

However, considerable evidence has been accumulated that raises serious doubts as to the sources of A, B and D genomes, but

Table 24.4. Sources of A, B and D genomes of *Triticum aestivum*. common hexaploid wheat.

Genome	*Source (diploid species)*	*Evidence*
A	*T. monococcum*	Pairing in F_1 from *T. aestivum* × *T. monococcum* and in F_1 from tetraploid wheats × *T. monococcum*.
B	*Aegilops speltoides*	Morphological features not contributed by A genome, chromosome morphology and chromosome pairing in interspecific hybrids.
D	*Ae. squarrosa*	Chromosome pairing in interspecific hybrids, morphology of the amphidiploid from *T. dicoccoides* × *Ae. squarrosa*, pairing in the hybrid from *T. aestivam* × the synthetic allohexaploid.

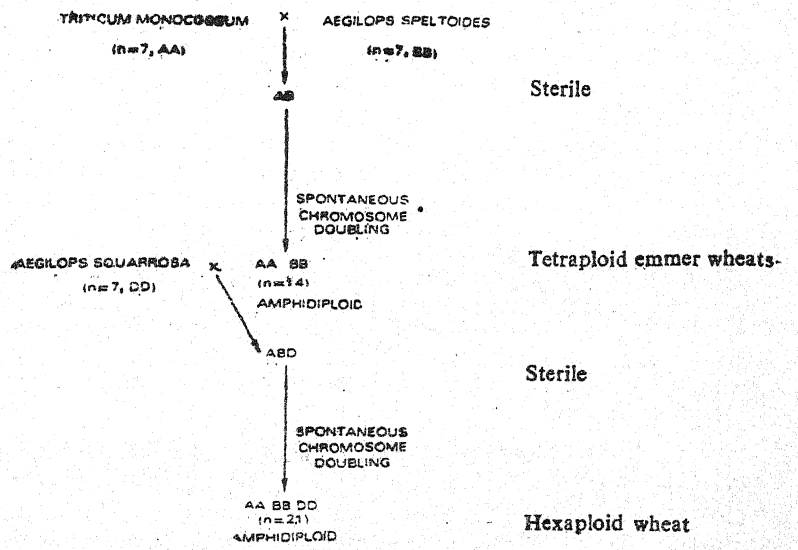


Fig. 24.5. A possible evolutionary history of hexaploid wheat.

at the same time there is no other hypothesis which is more plausible than the one outlined above. It is, therefore, concluded that the evolutionary history of wheat is, at best, not clear and often very confusing.

Evolution of *Nicotiana tobacum*. *N. tabacum* (*n*=24) is most likely an amphidiploid from the cross *N. sylvestris* × *N. tomentosa*; both the species are diploid with *n*=12. The interspecific hybrids *N. tabacum* × *N. sylvestris* and *N. tabacum* × *N. tomentosa* produce 12 II and 12 I at metaphase I. This indicates a homology between

chromosomes of *tabacum* and those of *sylvestris* and *tomentosa*. The amphidiploid from the cross *N. sylvestris* × *N. tomentosa* is similar to *N. tabacum* in many characteristics, which further supports the above conclusion. The species *N. tabacum* has undergone considerable differentiation during its evolutionary history, mostly due to the accumulation of gene mutations and, to some extent, due to the loss of some duplicated segments of the two gemones.

Evolution of *Gossypium hirsutum*. The 9 old World and the 8 New World species of *Gossypium* have $n=13$, but the chromosomes of the New World species are smaller than those of the Old World species. The other species, *G. hirsutum*, *G. barbadense* and *G. tomentosum* (wild Hawaii cotton), have $n=26$; in these species, 13 chromosomes are relatively larger than the remaining 13. A possible origin of *G. hirsutum* is from the cross between Asiatic cotton *G. arboreum* × *G. thurberi* (American wild cotton), followed by chromosome doubling of the interspecific hybrid. According to a more recent scheme, *G. hirsutum* has originated from the cross *G. herbaceum* var. *africanum* × *G. raimondii*, followed by chromosome doubling of the F_1.

Evolution of Amphidiploid ***Brassica*** **species.** The origin of amphidiploid *Brassica* species is presented in Fig. 24.6 based on the famous U's Triangle proposed by N. U in 1935. According to this scheme, *B. juncea* ($n=18$) is an amphidiploid from *B. nigra* ($n=8$) × *B. campestris* ($n=10$) ; *B. napus* ($n=19$) is an amphidiploid from the cross *B. oleracea* ($n=9$) × *B. campestris* ($n=10$), and *B. carinata* ($n=17$) is an amphidiploid from the cross *B. nigra* ($n=8$) × *B. oleracea* ($n=9$). The synthetic allopolyploids produced according to the above scheme resemble the natural amphidiploids, cross easily with them, and the hybrids between the synthetic and natural amphidiploids are reasonably fertile.

The determination of parental diploid species of the present-day allopolyploids presents many difficulties. Some of the major difficulties are listed below.

1. The parental diploid species may have become extinct, that is, they may not be present in the nature any more.
2. The parental diploid species may be expected to have differentiated considerably in comparison to when they entered the interspecific hybridization to produce the allopolyploid in question.
3. The allopolyploids themselves may have differentiated to a great extent from the state when they were first produced. Thus the genomes in allopolyploids may have changed to various degrees from those of the parental diploids.

In view of these difficulties, the evolutionary history of allopolyploids can be determined with only limited confidence ; it may not be possible to eliminate every doubt about the parentage of the present-day allopolyploids.

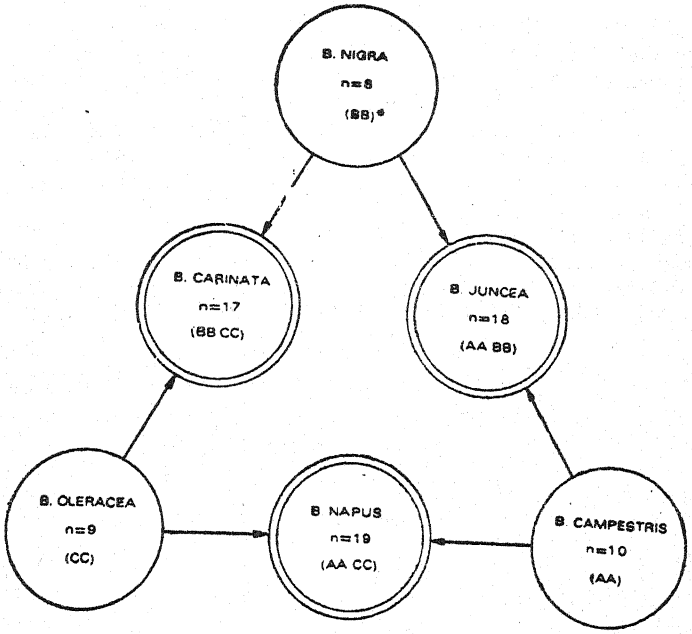


Fig. 24.6. The relationship between diploid and naturally occurring amphidiploid species of *Brassica*. The three diploid species are represented at the three tips of the triangle ; their amphidiploids are presented midway between the parental species (and are encircled by two concentric circles).
*Letters within parentheses denote the genomic symbols for diploid and amphidiploid species.

Applications of Allopolyploids in Crop Improvement

Allopolyploidy has three major applications in crop improvement : as bridging species in the transfer of characters from one species to another, in the production of new crop species, and in widening the genetic base of existing allopolyploid crop species.

Utilization As A Bridging Species. Amphidiploids serve as a bridge in the transfer of characters from one species to a related species, generally from a wild species to a cultivated species. The use of an amphidiploid as a bridging species becomes necessary when the hybrid between the cultivated species (recipient species) and the wild species (donor species) is sterile. The sterility of F_1 hybrid makes it impossible to cross the recipient species with the F_1, and this does not permit the transfer of the character from the donor to the recipient species. In such cases, the chromosome number of the F_1 interspecific hybrid is doubled to produce an amphidiploid which is generally reasonably fertile and can be crossed to the recipient

species. Progeny from the cross between the recipient species and the amphidiploid would have the somatic ($2n$) chromosome complement of the recipient species and one genome from the donor species. As a result, they would be sufficiently fertile to be used in backcross with the recipient species. From such a programme, alien addition and alien substitution lines are recovered, which are used in the transfer of genes, groups of genes or of small chromosome segments to the recipient species (Chapter 25).

An example of the use of an amphidiploid as a bridging species is the use of synthetic *N. digluta* (allohexaploid) for the transfer of resistance to tobacco mosaic virus from *N. sylvestris* to *N. tabacum* (Fig. 24.1). The F_1 hybrid from the cross *N. tabacum* × *N. sylvestris* is sterile. Chromosome doubling of the F_1 hybrid produces the synthetic allohexaploid *N. digluta*, which is reasonably fertile. *N. digluta* is backcrossed to the recipient species (*N. tabacum*) to produce a pentaploid having the complete somatic ($2n$) chromosome complement of *N. tabacum* and one genome of *N. sylvestris*. The pentaploid is sufficiently fertile to be backcrossed to *N. tabacum*. In the progeny, *N. tabacum*-like plants resistant to tobacco mosaic are selected and cytologically analysed. From the backcross progeny, both alien addition and alien substitution lines can be recovered.

Other examples of the use of an amphidiploid as a bridging species are in the cases of transfer of genes from *G. thurberi* to *G. hirsutum* and of chromosomes from *Haynaldia villosa* to *T. aestivum*. Many other examples of a similar use of an amphidiploid may be found in the literature. *For use as a bridging species, the features of the amphidiploid are not important ; it has to be only sufficiently fertile to be backcrossed to the recipient species.* Therefore, this application of synthetic amphidiploids is easy, immediate and more rewarding than the production of new crop species (in the short run).

Creation of New Crop Species. It was once hoped that allopolyploidy would enable man to create new species at will, and that these species would be superior to the existing crop species. This hope was based on the fact that some of the present-day important crop species are allopolyploids, and that the existing as well as new allopolyploids can be synthesized in the same manner as they would have been produced in the nature. Thus it was expected that a duplication of the nature's own methods would lead to the creation of new and superior crop species, as it occurred in the nature.

This hope, however, did not take into account the following facts : *first*, allopolyploidy itself has not enabled a species to become successful as a crop ; in fact, many allopolyploids are weedy wild species, *e.g.*, *S. spontaneum* and *S. robustum* are noxious weeds. *Secondly*, the natural allopolyploids have evolved over a long period to their present-day forms. Newly synthesized allopolyploids, therefore, could hardly be expected to become successful as crops. *Thirdly*, an allopolyploid that would be superior to the existing diploid

species would have already been produced and refined by the natural forces. Consequently, the allopolyploids that are not already existing may be expected to be inferior to the diploid species. These present a discouraging picture of the possibilities of using new allopolyploids as crop species, which seems to have been confirmed by the experience with synthetic allopolyploids.

Triticale is the most successful synthetic allopolyploid produced by crossing wheat (tetraploid or hexaploid) with rye (Fig. 24.4). Triticales derived from tetraploid wheats have been the most successful, but those from hexaploid wheats may also become a successful crop species. At present, triticales are being grown commercially in some parts of the world, *e.g.*, in Canada, and the yields of triticales are comparable to those of the best wheat varieties. The desirable features of triticales are that they combine the yielding ability and grain qualities of wheat and the hardiness (tolerance to adverse environment) of rye. But the development of such superior lines of triticales has taken 50 years of intensive research. The newly synthesized triticales were of low yielding ability due to high sterility, poor seed set and poor and variable development of grains. Triticales also show cytogenetic and genetic instability due to meiotic irregularities and produce some aneuploid progeny. In Sweden, the raw triticales yielded about 50 per cent of the standard varieties of wheat. The yielding ability of triticales increased under selection to about 90 per cent of the yield of wheat varieties in 15 years. Extensive breeding work on *Triticale* is going on at CIMMYT, Mexico. The strategy is to (1) produce a large number of triticale strains using different combinations (varieties as well as species) of wheat and rye, (2) hybridize these triticale strains, and (3) improve the defects of the triticales through selection. The results from such breeding programmes have been spectacular and have led to the release of several commercial varieties of *Triticale* which yield as much as the best varieties of common wheat.

Some other promising allopolyploids are *Raphanobrassica*, the triploid (AAC) obtained by crossing *B. napus* (AACC) with *B. campestris* (AA), allopolyploid clovers, *Festuca-Lolium* hybrids and some species hybrids in *Rubus* and Jute (*Corchorus sp.*). In *Rophanobrassica*, the breeding objectives are to combine the hardiness of *B. oleracea* with quick growth and disease resistance of fodder radish. The problems of *Raphanobrassica* are the same as those of triticales. *i.e.*, low fertility, cytogenetic and genetic instability and leafy rape-like plants that do not produce bulbs. There is evidence that hybridization and selection at the polyploid level would be effective in improving *Raphanobrassica*.

The amphidiploid *B. napus* (AACC) crosses very easily with *B. campestris* (AA) to produce the triploid (AAC) which has some desirable features. The triploid is produced so easily that it may be used as a hybrid variety, a special case of hybrid varieties produced by crossing two different species. Varalakshmi, a hybrid variety of

cotton is also an interspecific hybrid between *G. hirsutum* (American cotton and *G. barbadense* (Egyptian cotton).

Widening The Genetic Base of Existing Allopolyploids. The genetic base of some natural allopolyploids may be narrow, and it may be useful to introduce variability in such cases by producing new allopolyploids. *B. napus* is a case in point ; the genetic variability of this species is narrow and the only recourse available is to synthesize new allopolyploid *B. napus* to widen its genetic base. This is being done by crossing *B. campestris* (n=10, AA) with *B. oleracea* (n=9, CC), the parental diploid species, to produce the amphidiploid *B. napus* (n=19, AACC). The two species, *B. campestris* and *B. oleracea*, have to be crossed as autotetraploids; the cross is very difficult and embryo culture has to be used.

Limitations of Allopolyploidy

1. The effects of allopolyploidy cannot be predicted. The allopolyploids have some features from both the parental species, but these features may be the undesirable ones, *e.g.*, *Raphanobrassica*, or the desirable ones, *e.g.*, *Triticale.*
2. Newly synthesized allopolyploids have many defects, *e.g.*, low fertility, cytogenetic and genetic instability, other undesirable features etc.
3. The synthetic allopolyploids have to be improved through extensive breeding at the polyploid level. This involves considerable time, labour and other resources.
4. Only a small proportion of allopolyploids are promising ; a vast majority of them are valueless for agricultural purposes. Thus a costly trial and error has to be done before one is likely to come across a promising allopolyploid combination that can be improved through breeding to yield a new crop species.

SUMMARY

Changes in chromosome number (*heteroploidy*) may involve loss or gain of one or a few chromosomes (*aneuploidy*) or the whole genome (*euploidy*). A *trisomic* has one additional chromosome (*i.e* , $2n+1$) ; a *monosomic* has $2n-1$ and a *nullisomic* has $2n-2$. Aneuploids are generally weak and unstable cytogenetically ; monosomics and nullisomics survive only in polyploid species. Aneuploids occur spontaneously, may be produced in high frequencies from triploids, autotetraploids, desynaptic plants, tetrasomic plants and translocation heterozygotes. Aneuploids are useful in study of the effects of loss or gain of a chromosome, in locating linkage groups and genes in a particular chromosome or even in a chromosome arm, in chromosome substitution and in establishing homoeology between genomes of an allopolyploid species. Aneuploid analysis suffers from the difficulty in production and maintenance of aneuploid lines and the necessity for extensive cytological analysis.

In euploids, the same genome may be present more than twice (*autopolyploidy*) or there may be two or more distinct genomes (*allopolyploidy*). Autopolyploids are produced spontaneously, by gamma irradiation, from tissue culture and by treatment with some chemicals of which colchicine is the most successful and widely used. Autopolyploids are generally larger in size, have

larger cells, stomata, pollen grains and seeds, are slower in growth, later in flowering and maturity, have thicker leaves, show high sterility and multivalent formation. Segregation in autopolyploids is very complex. Some of our crops are autopolyploids, *e.g.*, potato, coffee, sweet-potato, groundnut, alfalfa and banana. Autopolyploidy has the following applications in crop improvement : *monoploids* are useful in production of homozygous lines and may be useful in studies of mutation. *Triploid* watermelon is seedless and hence desirable, while triploid sugarbeet is high yielding. *Tetraploids* may be useful in breeding, *e.g.*, in banana, in production of interspecific hybrids, *e.g.*, *B. campestris* × *B. oleracea* (both must be $4n$) and *B. oleracia* ($4n$) × *B. chinensis*, and directly as crop varieties, *e.g.*, Double Steel and Tetra Petkus, Giant Berseem etc. The limitations of autopolyploidy are higher water content of autopolyploids, high sterility requiring extensive breeding, difficulties in maintaining autopolyploids due to cytogenetic and genetic instability, and the inability to predict the effects of autopolyploidy.

Allopolyploids are produced by chromosome doubling of interspecific hybrids (*amphidiploid*). Chromosome doubling may occur in somatic tissues or may result from production of unreduced gametes which unite to produce an amphidiploid. Chromosome doubling is generally induced by colchicine. Allopolyploids combine some characteristics of each parental species, often are hardier than parental diploids, some show apomixis, almost all new allopolyploids show variable sterility, often show cytogenetic and genetic instability and a variable number of multivalents at meiosis. Fertility and bivalent formations are easily improved by selection. Naturally evolved allopolyploids show only bivalent formation since they have developed genetic systems that prevent homoeologous pairing. *e.g.*, 5B system in common wheat. Allopolyploidy has played an important role in evolution of plants ; some of the important allopolyploid crops are wheat, oats, cotton, tobacco, some *Brassica* species etc. The most probable evolutionary history of many of the allopolyploid corps has been determined. Allopolyploidy is useful in crop improvement since the synthetic allopolyploids may be used as a bridging species in gene transfer from a wild species to a cultivated one, as new crop species, *e.g.*, *Triticale*. *Raphanobrassica* etc. and as a source of new genetic variation in an existing allopolyploid. *e.g* , synthetic *B. napus*, for widening the genetic base of natural amphidiploid *B. napus*. The limitations of allopolyploidy are that its effects cannot be predicted, raw allopolyploids have many defects which have to be improved through breeding involving considerable resources, and only a small proportion of allopolyploids are of any promise.

QUESTIONS

1. Define heteroploidy. List the various types of changes in chromosome number. Briefly describe the modes of origin of aneuploids and their applications in crop improvement.
2. What are the common aneuploid analyses for locating genes on a particular chromosome ? Give a brief description of these techniques using suitable diagrams where necessary.
3. Define autopolyploidy. Briefly describe the methods of production of autopolyploids and their importance and limitations in crop improvement.
4. What is allopolyploidy? Discuss the origin in nature and experimental production of allopolyploids.
5. Briefly discuss the evolution of following crops : (i) *Brassica* sp., (ii) *Triticum aestivum*, (iii) *Nicotiana tabacum*, and (iv) *Gossypium hirsutum*.
6. With the help of suitable examples, discuss the applications and limitations of allopolyploidy in crop improvement.
7. Differentiate between the following : (i) haploids and monoploids, (ii) double trisomic and tetrasomic, (iii) Homologous and homoeologous chromosomes, (iv) autopolyploid and allopolyploid, (v) diploid and diploidized species, (vi) aneuploid and euploid, and (vi) polyploidy and heteroploidy.

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CHAPTER 25

Distant Hybridization in Plant Breeding

In crop improvement programmes, the parents used in hybridization are generally different varieties of the same species. In such cases, hybridization and production of subsequent generations does not present any problem (except for self-incompatibility and male sterility systems). Crosses between varieties of the same species represent the vast majority of crosses used in crop improvement. But in many cases, it may be desirable, or even necessary, to cross individuals belonging to two different species or genera. When individuals from two distinct species of the some genus are crossed, it is known as *interspecific hybridization.* When individuals being crossed belong to species from two different genera, it is referred to as *intergeneric hybridization.* An example of interspecific hybridization is a cross between the cultivated rice (*Oryza sativa*) and the wild rice (*Oryza perennis*), while intergeneric hybridization may be illustrated by a cross between wheat (4*x* or 6*x Triticum*) and rye (*Secale cereale*). *Hybridization between individuals from different species, belonging to the same genus or to different genera, is termed as distant hybridization ; such crosses are known as distant crosses or wide crosses.* This is because individuals used for hybridization in such cases are taxonomically more distantly related than different varieties from the same species.

HISTORY OF DISTANT HYBRIDIZATION

The first authentic record of a distant hybridization for crop improvement is the production of a hybrid between carnation (*Dianthus caryophyllus*) and sweetwillian (*Dianthus barbatus*) by Thomas Fairchild in 1717. Subsequently, a large number of interspecific hybrids were produced. Most of the interspecific hybrids were of no agricultural value, and were of academic interest only. But many interspecific hybrids, particularly in case of ornamentals, served as commercial varieties. An interesting intergeneric hybrid, *Raphanobrassica*, was produced by a Russian scientist named Karpechenko in 1928. *Raphanobrassica* is the amphidiploid from a cross between radish (*R. sativus*) and cabbage (*B. oleracea*). *Raphanobrassica*, was produced with a view to combine the root of radish with the leaves of cabbage, but it had roots like cabbage and leaves like radish ! a useless combination of characters. But the first

intergeneric hybrid, and with a greater potential than *Raphanobrassica*, was *Triticale*. *Triticale* was first produced by Rimpau in about 1890. *Triticale* is the amphidiploid obtained from crosses between wheat and rye. About 50 years of intensive research has improved the characteristics of triticales considerably ; it is beginning to become an accepted crop in certain areas, *e.g.*, Canada. At present, distant hybridization has a definite role to play in crop improvement programmes : improvement of a few crop species is based on interspecific hybridization, *e g.*, sugarcane (*S. Officinarum*), but in most of the cases it is used for the transfer of a few desirable genes, particularly those for disease resistance. In many crop species, the genes for disease resistance transferred from the wild species are extremely useful. Jack Harlan concluded that 'there are several examples in which genes from wild relatives stand between man and starvation or economic ruin.'

BARRIERS TO PRODUCTION OF DISTANT HYBRIDS

The difficulty in the production of distant hybrids varies to a great extent. In some cases, distant hybrids may be obtained without an appreciable difficulty, and hybridization may be as easy as intervarietal hybridization, *e.g.*, cultivated tomato (*Lycopersicon esculentum*) crosses easily with the closely related species (L. *pimpinellifolium*). But in a vast majority of cases, F_1 hybrids may be obtained with variable degrees of difficulty, and in many cases hybrids may not be obtained with the currently available techniques, *e.g.*, cabbage (*B. oleracea*) does not hybridize with the closely related turnip (*B. rapa*) or rape seed (*B. napus*). In general, taxonomic relationship is a fair guide of the ease with which distant hybrids may be obtained : closely related speceis may be expected to cross more easily than those more distanly related. However, this general relationship is only an indication ; specific cases differ to a great extent and the experimenter has to find for himself how easily the two species he desires to cross will actually hybridize.

The difficulties encountered in the production of interspecific hybrids may be grouped into three broad classes : failure of zygote formation, failure of zygote development and failure of F_1 seedling development A variety of mechanisms may be responsible for each of these three difficulties ; the more common barriers are briefly described below.

Failure of Zygote Formation. In many cases, distant hybrids are not produced because the zygote is not formed due to the failure of fertilization. Fertilization often fails because the pollen tube is unable to reach the embryo sac and consequently the sperms are not available for fertilization. The pollen tube may burst in the style of another species and thus fail to reach the embryo sac ; this occurs in some species combinations in *Datura*. In some cases, the style of the

species used as female is longer than the pollen tube would normally grow. Therefore, the pollen tube does not reach the embryo sac, *e.g.*, in crosses between maize (*Z. mays*, ♀) and *Tripsacum* sp. (♂). In such cases, the cross may be made by using the species with the shorter style as female or by cutting off a portion of style of the parent with the longer style. Pollen tubes of polyploid species are usually thicker than those of diploid species. When a diploid species is used as female and a polyploid one as male, the polyploid tube grows at a slower rate in the diploid style than it would in a polyploid style. This difficulty may be overcome by using the diploid species as the male parent or by shortening the style of the diploid species being used as female. These are some of the more common barriers to zygote formation ; other barriers may be encountered in specific cases.

Failure of Zygote Deveplopment. In many cases, fertilization does take place, and zygote is produced. But the development of zygote is blocked at various stages. Failure of embryo development may take place due to lethal genes, genotypic disharmony between the genomes of the two parental species, chromosome elimination, cytoplasmic incompatibility and endosperm abortion.

Lethal Genes. Some species carry a lethal gene which causes death of the zygote during early embryonic development. The lethal gene generally does not have any effect in the species carrying it but affects only interspecific hybrid embryos. For example, *Aegilops umbellulata* has a gene for lethality that has three alleles : L^e for early lethality, L^l for late lethality and l for nonlethality. This lethal gene is active against the zygotes produced by crossing *Ae. umbellulata* with diploid ($2x$) wheats. Similarly, *Crepis tectorum* has a lethal gene l which causes death of the embryo in the early stages of development in certain interspecific crosses. Interspecific hybrids in such cases may be obtained by using a strain (of the species that carries the lethal gene) carrying the nonlethal allele, *e.g.*, l in *Ae. umbellulata*, and L in *C. tectorum*, for making the interspecific crosses.

Genotypic Disharmony between The Two Parental Genomes. In some cases the death of embryo may occur due to genetic imbalance rather than the action of specific lethal genes. There is a possibility that cases of genotypic disharmony may be cases of lethal gene action as well but due to insufficient genetic analysis the lethal genes have not been detected. A supposed case of genotypic disharmony occurs in interspecific crosses in cotton involving *Gossypium gossypioides* as one of the parents. The hybrids from these crosses die in various stages of development ranging from early embryonic development to the reproductive phase of the F_1 plants. Crosses with *G. davidsoni* also show early embryonic mortality. But lethal genes are known in other interspecific crosses in cotton. In seems likely that the above cases are also due to lethal genes, but there is no clearcut evidence for it.

When the failure of zygote development is due to genetic inbalance in the hybrid, interspecific hybrids cannot be obtained since they would not survive owing to genetic disturbances. But if such cases are complicated cases of lethal gene action, interspecific hybrids may be obtained by using strains that do not have the lethal allele. Therefore, in cases where genetic imbalance seems to be the reason for the failure of zygote development, several strains of each species should be crossed with each other. If some combinations produce viable progeny, while others do not, the case is most likely one of lethal gene action. Different genotypes/strains of a species show considerable variation in ease with which they produce interspecific hybrids ; the genetic basis and the mechanism of this variation is not precisely known.

Chromosome Elimination. In some cases of distant hybridization, chromosomes are gradually eliminated from the zygote. This generally does not prevent embryo development, but the resulting embryo and the F_1 plants obtained from them are not true interspecific hybrids since they do not have the two parental genomes in full. Generally, chromosomes from one genome are successively eliminated due to mitotic irregularities, and in extreme cases, chromosomes of only one genome may remain in the embryo. Such embryos are, consequently, haploid. Chromosome elimination has been reported in certain interspecific crosses in *Nicotiana* and *Hordeum*, and in crosses between *Triticum aestivum* (♂) and *Hordeum bulbosum* (♀). When *Hordeum vulgare* or *T. aestivum* are used as male and *H. bulbosum* is used as female, the embryos obtained from such crosses are generally haploid carrying the haploid genomes of *H. vulgare* and *T. aestivum*, respectively. In fact, this cross is the basis for large scale haploid production programmes in barley.

Incompatible Cytoplasm. In a few cases, embryo development may be blocked by an incompatibility between cytoplasm of the species used as female and the genome of the species used as male. Such an interaction, more generally, leads to hybrid weakness and male sterility in the hybrids. But in a few cases, embryo development may also be prevented.

Endosperm Abortion. Seeds from a large number of distant crosses are not fully developed and are shrunken due to poorly developed endosperm. Such seeds show poor germination, and may often fail to germinate. When endosperm development is poor or is blocked, the condition is generally known as *endosperm abortion.* The endosperm may abort during early embryo development preventing further development of the embryo, or it may abort at a later stage allowing an almost complete development of the embryo. Examples of endosperm abortion are common in distant hybridization. A familiar example is the cross between *Triticum* and *Secale* to produce *Triticale.* In this case, the endosperm aborts at a much later stage so that a small frequency of viable seeds is obtained. But in the cross *H. bulbosum* × *H vulgare*, the endosperm aborts at an early stage so that viable seeds are not produced.

In case of endosperm abortion, hybrid plants may be raised by culturing the hybrid embryo on a suitable culture medium (Chapter 26). The embryos, in such cases, are removed from young seeds before endosperm abortion takes place.

Failure of Hybrid Seedling Development. Some distant hybrids die during seedling development or even after initiation of flowering. Some interspecific hybrids in *Melilotus* are chlorophyll deficient and fail to survive. Such plants may be grown to maturity by grafting them on to normal plants or by growing them in vitro. Certain interspecific hybrids in cotton appear normal, but die in various stages of seedling growth ; some plants die after flowers have been formed. The mechanisms involved in the failure of seedling development may be expected to be of a similar nature as those involved in the failure of embryo development. Lethal genes, genetic imbalance and cytoplasmic incompatibility may be involved.

TECHNIQUES FOR PRODUCTION OF DISTANT HYBRIDS

Many interspecific hybrids may be produced if a sufficiently large number of flowers are pollinated. However, in many cases, hybrid seeds may not be obtained inspite of a large number of pollinations. The next step consists of determining the barrier to production of hybrid embryos and then using measures to overcome these barriers. In general, the species with shorter style should be used as the female parent. Where this is not possible or desirable, a part of the style of the other parent may be cut off to make it shorter, *e.g.*, part of the stigma of maize is cut-off when it is crossed as female with *Tripsacum*. Different strains within a species show considerable variation in success in interspecific hybridization, particularly when lethal genes are involved. It may be desirable to attempt hybridization with more than one strain of each of the parental species. In some cases, autopolyploidy may be helpful in interspecific hybridization. *B. oleracea* (cabbage) and *B. campestris* (turnip rape) do not cross with each other at the diploid level, but they produce embroys when tetraploid forms of the two species are crossed; embryo culture has to be used for the recovery of embryos. Environment also influences the success of hybridization. If possible, the crosses may be attempted under different environmental conditions.

When species with different ploidy levels are crossed, hybridization between them is relatively more difficult than when species with the same ploidy level are mated. In such a case, one of the following four approaches may be useful. *Firstly*, they may be crossed directly; in such a case, the species with the higher ploidy level is generally used as the female parent. *Secondly*, the chromosome number of the wild species or of the interspecific hybrid (F_1) may be doubled to overcome sterility of the hybrid. Many wild species of potato have been crossed with *S. tuberosum* after their

chromosome numbers were doubled, while the latter approach has been used in tobacco. In the *third* approach, chromosome number of the polyploid species is doubled to obtain a semisterile interspecific F_1, which can be either self-pollinated or backcrossed to the cultivated species, *e.g.*, *8x N. tabacum* × *N. otophora*. *Fourthly*, the chromosome number of the higher ploidy level species may be reduced. This is more difficult than increasing the chromosome number, but in potato certain combinations of seed parents produce 35-80% dihaploid ($n=2x$) progeny. This approach has been extensively used in potato.

When two species, say A and C, cannot be crossed directly, a third species, *e.g.*, B, may be used as a *bridge species*. The bridge species must be compatible, *i.e.*, able to cross, with both the species (A and C) which are to be crossed. In order to transfer genes from C to A, C is first mated with B. The resulting hybrid or its amphidiploid is then crossed with species A. An example of a bridge cross is *Aegilops ventricosa* × *Triticum turgidum*; the F_1 from this cross was crossed to *T. aestivum*, which produces sterile hybrids with *Ae. ventricosa*. This technique was used to transfer resistance to eye spot from *Ae. ventricosa* to *T. aestivum*. Bridging crosses have mostly been used in wheat and tobacco. Use of bridging species (1) is complicated, (2) selection for the desirable genes is often difficult, and (3) dominant monogenic characters are perhaps the only ones whose transfer is feasible.

Many interspecific crosses, hitherto not possible, were successful due to the use of growth regulators, *e.g.*, IAA, 2,4-D, naphthalene acetamide etc. *N. tabacum* does not hybridize with *N. repanda*, but this cross is possible when IAA is applied to the pedicel of flowers in a lanoline paste. Similarly, application of 2, 4-D prior to pollination, followed by gibberellic acid treatment has permitted intergeneric crosses between *Hordeum vulgare* and species of *Avena*, *Phleum*, *Dactylis*, *Triticum*, *Lolium* and *Festuca*. In some cases, use of an immunosuppressant may be quite useful, *e.g.*, reciprocal crosses between *Vigna radiata* and *V. umbellata* were successful when EACA (E-amino caproic acid) was applied to flower buds before pollination.

In many cases, failure of seed set in interspecific crosses is due to endosperm abortion. This may be overcome by using the embryo culture technique. Embryo culture is routinely employed in making many distant crosses, *e.g.*, cross between *Triticum* and *Secale*.

STERILITY IN DISTANT HYBRIDS

Distant hybrids show variable sterility ranging from complete fertility to complete sterility. An example of almost complete fertility is provided by the hybrid between *L. esculentum* (tomato)

and *L. pimpinellifolium*, and that of complete sterility by the sugarcane-maize hybrid produced at the Sugarcane Breeding Institute, Coimbatore, and being maintained by clonal propagation. Distant hybrids may be classified into two broad groups. The *first group* includes those distant hybrids that exhibit at least some fertility so that they can be maintained by selfing, intercrossing among themselves or backcrossing to the parental species. The *second group* consists of those hybrids that are completely sterile and have to be maintained clonally or by doubling their chromosome number; the latter increases the fertility and seed set in the amphidiploids thus obtained. The sterility of distant hybrids may be caused by cytogenetic, genetic or cytoplasmic factors.

Cytogenetic Basis of Sterility. Most of the interspecific hybrids show reduced pairing ; in extreme cases, all the chromosomes may be present as univalents. The distribution of chromosomes in such cases is irregular, and it leads to the formation of unbalanced gametes resulting in partial to complete sterility. Interspecific crosses also show rings and chains at metaphase I (indicating translocations), bridges and fragments at anaphase I (indicating inversion) and loops at pachytene (indicating duplication or deletion). These cytological aberrations also reduce fertility wherever they occur. Fertility in such hybrids is improved by doubling their chromosome number, that is, by producing amphidiploids from them.

Stebbins demonstrated that in many interspecific hybrids sterility may result from small structural changes in chromosomes. These structural changes would not affect chromosome pairing to any appreciable degree and would not be detectable at metaphase I. Stebbins calls these small chromosomal changes *cryptic structural changes*. Sterility due to cryptic structural changes would be difficult to distinguish from cases of genetic sterility.

Genetic Basis of Sterility. Chromosome pairing in some interspecific hybrids is regular, but they show variable sterility. It has been suggested that sterility in such cases is due to genes rather than structural chromosome changes or reduced pairing. For example, F_1 hybrid between foxtail millet, *Setaria italica*, and its wild relative *S. viridis* showed normal pairing and regular formation of 9 bivalents. But pollen and ovule sterilities were 70 and 50 per cent, respectively. Hybrids between varieties of the two subspecies of cultivated rice (*O. sativa*), *indica* and *japonica*, show variable sterility. Oka showed that several genes were responsible for sterility in *indica*×*japonica* hybrids ; these genes acted in complementary fashion. But there is evidence that the genomes of *indica* and *japonica* rices have differentiated to some extent in terms of chromosome structure. Therefore, it is possible that the genes affecting gametophyte development, *i.e.*, sterility in the hybrids, may, in fact, be small structural changes in chromosomes.

It is very difficult to conclusively show the existence of sterility in interspecific hybrids to be controlled by genes. It is likely that

most cases of supposed genic sterility are due to small chromosome changes. Such changes would produce in the interspecific hybrids what Stebbins terms as *cryptic structural hybridity*, that is, heterozygosity for small chromosomal rearrangements, duplications and deficiencies.

Cytoplasmic Basis of Sterility. In some interspecific hybrids, sterility is produced by the cytoplasm. In such cases, the reciprocal crosses produce fertile hybrids, that is, fertility of the hybrid depends upon which of the two species was used as female. Hybrids between certain races of *Culex pipiens* mosquitoes show reciprocal differences in fertility, *i.e.*, the hybrid is fertile when one race is used as female, but it is sterile when the other race is used as female. Clearly, in such cases sterility is produced by the cytoplasm. Such instances of hybrid sterility are also known in plants, *e.g.*, *Epilobium*, *Oenothera* etc.

CONSEQUENCES OF SEGREGATION IN DISTANT HYBRIDS

Distant hybrids involve two distinct species belonging to the same genus or to two different genera. These species would differ for a large number of genes. Therefore, distant hybrids would be heterozygous for many genes ; the number of heterozygous genes would be several times the number involved in intervarietal crosses. *Segregation, as a result, would produce a very wide range of phenotypes in F_2 and the later generations. Many of the segregants would have entirely new characteristics distinct from those of the two parental species.*

The two species entering a distant cross are expected to possess somewhat different character combinations of adaptive significance. Evolution and natural selection would have preserved and developed those character combinations in these species that make them well adapted to their environments. When these characters are recombined at random, most of the new character combinations are likely to be much inferior to the parental combinations. *As a result, a majority of segregants from interspecific hybrids are much weaker and less adapted than the parental species.*

The range of recombinants recovered from the segregating generations of interspecific hybrids is quite extensive. *But the recombinants recovered represent only a small proportion of the total recombinants that are possible. Thus recombination in interspecific hybrids produces only a limited range of variability from the total range possible.* The possible causes of restricted range of recombinants are gametic elimination, zygotic elimination and linkage.

Interspecific hybrids generally show reduced pairing ; in extreme cases, no bivalents may be formed. The reduced pairing is due to chromosome differentiation and shows a lack of homology between the chromosomes of the two species. In such cases, the distribution of chromosomes at anaphase I of meiosis is not precise

and many unbalanced gametes are produced. Even where pairing does occur, crossing over, *i.e.*, recombination between linked genes, is drastically reduced. *Therefore, segregation for different characteristics does not fit the Mendelian ratios in F_2's from wide crosses. This may be due to irregular meiosis, impaired recombination and reduced survival of many recombinant gametes and zygotes.*

The male gametes are more sensitive to chromosomal, genetic or environmental factors than the female gametes. As a result, many distant hybrids show a very high male sterility and they have to be backcrossed with one of the parental species which serves as the male parent in the backcross. *But even when the F_1 is partially fertile, the F_2 is more like the backcross progeny than a true F_2 generation.* This is because the male gametes that are most likely to survive are those that have the same chromosome complement as that of one of the parental species or very close to it. In an extreme case, the surviving male gamete may be identical with one of the parental species. For example, the surviving pollen from F_1 of the cross. *T. aestivum* × *T. timopheevii* is almost exactly like that of *T. timopheevii*. However, in many cases, male gametes that are close to, but not necessarily identical with the parental species in their chromosome complements do survive and the F_2 shows a much wider range than the backcross.

APPLICATIONS OF DISTANT HYBRIDIZATION IN CROP IMPROVEMENT

Many of our important crop species are allopolyploids, *e.g.*, wheat, oats, sugarcane, cotton etc. These crops evolved through distant hybridization, followed by spontaneous chromosome doubling of the nearly sterile F_1 hybrids. The chromosome doubling may have taken place in somatic tissues due to mitotic irregularities producing an amphidiploid sector in the plant. Alternatively, unreduced gametes may be produced due to meiotic irregularities ; when two such gametes unite, an amphidiploid progeny would be produced. Colchicine gave man a tool to double the chromosome number of plants at will, and it was once thought that many new and useful crop species would be developed through distant hybridization, followed by chromosome doubling of the interspecific hybrids. The success of this approach was taken for granted since the method was only to duplicate the process that had already been so successful in the nature. But experience has had a sobering effect. Creation of new crop species is still one of the major objectives of distant hybridization, but it has found several other, and perhaps more immediate, applications in crop improvement; *e.g.*, alien addition lines, alien substitution lines, gene transfers, transfer of cytoplasm and, in a few cases, use as new crop varieties.

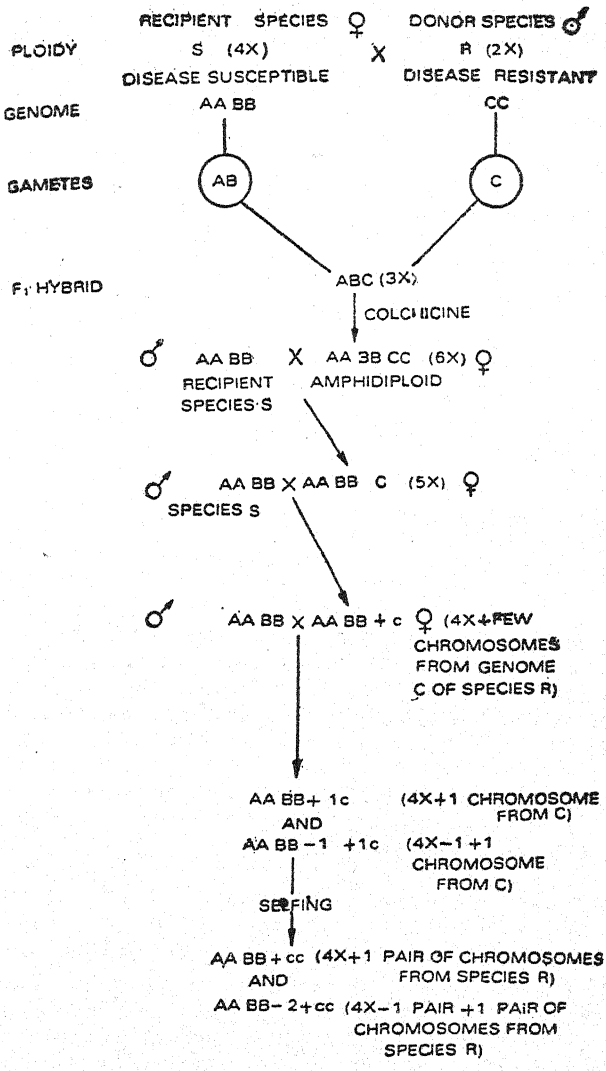
Alien-Addition Lines. *An alien-addition line carries one chromosome pair from a different species in addition to the normal diploid chromosome complement of the parent species.* When only one chromosome (not a pair of chromosomes) from another species is present,

it is known as *alien-addition monosome.* Alien addition lines have been produced in wheat, oats, tobacco and several other species. The purpose of alien-additions generally is the transfer of disease resistance from related wild species, *e.g.*, transfer of mosaic resistance from *Nicotiana glutinosa* to *N. tabacum.* This addition line had the full diploid complement of *N. tabacum* ($2n$=48) plus a pair of H chromosome carrying mosaic resistance from *N. glutinosa* ($2n$= 24) ; thus the *somatic* ($2n$) chromosome number of the alien-addition line was 50.

Alien-addition lines are of little agricultural importance since the alien chromosome generally carries undesirable genes. For example, the addition line in tobacco, mentioned above, had reduced growth and short broad leaves in addition to mosaic resistance.

Alien-Substitution Lines. *An alien-substitution line has one chromosome pair from a different species in the place of one chromosome pair of the recipient species. Alien-substitution monosome* has a single chromosome (not a pair) from a different species in place of a single chromosome of the recipient species. Alien-substitutions have been developed in wheat, cotton, tobacco, oats etc. In case of tobacco, mosaic resistance gene *N* was transferred from *N. glutinosa* to *N. tabacum* ; the resistant *N. tabacum* line had 23 pairs of *tabacum* chromosomes and one pair (chromosome H) of *glutinosa* chromosomes. The alien-substitutions show more undesirable effects than alien-additions, and as a consequence are of no direct use in agriculture.

Both alien-additions as well as substiutions are effectively viable in polyploid species only. Consequently, they are confined to polyploid crop species, such as, wheat (*T. aestivum*), tobacco (*N. tabacum*), cotton (*G. hirsutum*), oat (*A. sativa*), potato (*S. tuberosum*) etc. Substitution and addition lines are produced through the backcross method. The *recurrent parent species* (in which the alien chromosome is to be added or substituted) is crossed with a related *donor species* (from which the chromosome is to be added or substituted). In the example illustrated in Fig. 25.1, the recurrent parent species S is susceptible to a disease, while the donor species R has a gene for resistance to this disease. The resulting interspecific hybrid is made amphidiploid by chromosome doubling using colchicine. The amphidiploid thus obtained is backcrossed to the recurrent parent species (species S in Fig. 25.1) to produce a progeny carrying the full somatic ($2n$) chromosome complement of the recurrent species S (AA BB) and a single genome of the donor species R (C). This is then backcrossed to the recurrent species S ; the resulting progeny have the full somatic complement of the recurrent species S and a variable number of chromosomes from the donor species R. Resistant (to the concerned disease) plants are selected and may be backcrossed to the species S. Resistant plants carrying the somatic ($2n$) chromosome complement of species S and one additional chromosome are selected ; these are *alien-addition monosomes.* These plants would show a single univalent at meiotic metaphase I. Alien-



Hybridize the two species R and S

F_1 is usually sterile. Chromosome number is doubled by colchicine.

The amphidiploid thus produced is fertile. It is back-crossed to the recipient species S.

The progeny is a pentaploid with a single C genome from the species R and the full diploid complement of species S.

The progeny have full somatic complement of S and a few chromosomes from R. Disease resistant plants selected and backcrossed to species S.

Disease resistant plants similar to species S selected. Alien-addition monosome has one extra chromosome; substitution monosome has the $2n$ number of S.

$2n+2$ disease resistant plants are alien-addition lines. Resistant plants with $2n$ chromosome are alien-substitution lines

Fig. 25.1. Production of alien-addition and alien-substitution lines. A simplified scheme based on two hypothetical species S and R; a chromosome from species R carrying a disease resistance gene is to be added or substituted in the full somatic ($2n$) chromosome complement of species S, which is susceptible to the disease.

addition monosomes are selfed to isolate resistant plants carrying one chromosome pair in addition to the full somatic complement of the species S ; these are *alien-addition lines. The hybrid between an alien-addition line and the recurrent parent species S would show a single univalent at metaphase I.*

Some resistant plants would have the somatic chromosome number of the recurrent species S. That is, these plants would carry the gene for resistance, but would not carry an extra chromosome. At metaphase I, such plants may show 2 univalents if one chromosome of the recurrent species S was replaced by one chromosome of the donor species R. These are *alien-substitution monosomes.* The alien-substitution monosomes are selfed to obtain resistant plants with the same chromosome number as that of the recurrent species S, but producing regular bivalents during meiosis. These plants would be *alien-substitution lines. Two univalents would be seen at metaphase I of the hybrid between the recurrent species S and the alien-substitution line.*

Transfer of Small Chromosome Segments. Transfer of small chromosome segments carrying specific desirable genes has been widely used in crop improvement programmes. It would be ideal to transfer only the desired gene from the related alien species, since the gene under transfer may be linked with other undesirable genes. Therefore, transfer of small chromosome segments is limited in its usefulness because of this problem. In some cases, the interspecific hybrid may be sufficiently fertile and can be backcrossed with the species to which the gene is being transferred. In such cases, the homology between the chromosomes of the two species may be enough to allow recombination between the chromosomes of the donor and the recipient species. By backcrossing the interspecific F_1 hybrid to the recipient species, resistant plants may be isolated which have the gene for resistance incorporated into the chromosome of the recipient species by recombination. An example of such a gene transfer is the transfer of black arm resistance from *G. barbadense* (Egyptian cotton) to *G. hirsutum* (American upland cotton). The interspecific hybrid *G. barbadense* × *G. hirsutum* is fertile, and upon backcrossing to *G. hirsutum* produced *hirsutum*-like plants resistant to back arm.

In a large number of cases, however, alien-addition or substitution monosomes are produced since the interspecific hybrid is not fertile. The transfer of gene for resistance is accomplished in three ways : spontaneous crossing over, promotion of homoelogous pairing and crossing over, and induction of translocations using X-rays or gamma-rays. (1) In many cases, *spontaneous crossing over* occurs between the alien chromosome and a chromosome of the recipient species. Thus the gene under transfer is transferred to the chromosome of the recipient species. For such transfers, alien substitution monosomes are generally employed. (2) When spontaneous crossing over does not occur due to infrequent pairing between the alien chromosome and the chromosome of the recipient species, *pairing may be promoted* by certain genetic/cytogenetic manipulations. In

T. aestivum chromosome 5B carries a gene, *Ph*, that prevents pairing between homoeologous chromosomes, *e.g.*, chromosomes 5A, 5B and 5D. Pairing with the alien chromosome may be promoted by using in the interspecific hybridization a wheat line in which the chromosome 5B lacks the homoeologous pairing suppressor (*i.e.*, has the mutant *Ph* allele in homozygous state), or which is nullisomic for 5B. Such a technique is useful where the knowledge of the genetic control of chromosome pairing is sufficiently advanced and suitable genetic stocks are available. (3) An alternative technique of a more general applicability is to induce translocations using X-rays or gamma-rays. Many transfers have been made by this method, notably in wheat and tobacco. But translocations are generally less desirable than spontaneous or promoted recombinations since they tend to transfer chromosome segments that may carry undesirable linked genes. Consequently, many of the gene transfers produced by translocations are unsatisfactory for use in the crop improvement. Currently, a technique is being developed in wheat for using such translocations for isolating lines that have received the gene under transfer from the alien chromosome segment through recombination.

The techniques used for promoting homoeologous pairing and translocation are known as *chromosome manipulation techniques*. It may be pointed out that in most of the cases, interspecific gene transfers have been made by spontaneous recombination. Use of chromosome manipulation techniques may often not be necessary, and they require considerable cytological analysis and are time consuming.

Genes affecting a variety of characters have been transferred from wild relatives ; these include disease resistance, wide adaptation, improved quality, mode of reproduction, yield etc.

1. **Disease Resistance**. Disease resistance is by far the most common characteristic transferred to the cultivated species from their wild relatives. Some important examples are, rust resistance in wheat; late blight resistance in potatoes ; resistances to bacterial wilt, bacterial canker, curly top virus, mosaic virus etc. in tomato; resistance to many insects and diseases in sugarcane and so on. There is hardly any crop species which has not benefited from such a gene transfer. In some cases, the contribution of gene transfers has been so great that it prompted Harlan to observe that 'some crops could not maintain commercial status without genetic support of their wild relatives'. A glaring example is the potato famine in Europe during 1840s caused by late blight ; about one million Irish died as a result of this famine. *Solanum demissum* provided genes for late blight resistance, which served as a miracle saver of the potato crop, although only temporarily.

2. **Wider Adaptation**. Wild species have served as useful sources of genes for earliness and wider adaptation. *Cold tolerance* has been transferred from wild relatives to wheat, onion, potato, tomato, grape, rye and peppermint. *Earliness* has been transferred

to cultivated species of *Brassica* and soybean from their wild relatives. Wild species of wheat and peas have contributed genes for *drought and heat tolerance.* Other similar gene transfers are : *salt tolerance* to tomato, *tolerance to calcareous soils* in grape, and *lack of photosensitivity to Penntisetum.*

3. **Quality.** There are some notable instances where quality of a crop has been improved by the genes transferred from its wild relatives. Genes for increased protein content have been transferred to rice, soybean, oats and rye. The high protein content of wild species is generally associated with small seed size : a selection for increased seed size during gene transfers generally leads to a reduction in the protein content to a level comparable to that of the cultivated species. The above mentioned examples are notable in that they have been achieved in spite of this difficulty. Oil quality in oil palm has been improved by genes from its wild relatives. Other examples of genes for improved quality from wild relatives are : increased soluble solids (from green-fruited species) and carotenoid content (the *B* gene from *L. hirsutum*) in tomato ; improved leaf quality in tobacco (from *N. debneyi*) ; and improved fiber strength in *G. hirsutum* (from the lintless *G. thurberi* ; the allotetraploid from the cross *G. thurberi* × *G. arboreum* was crossed with *G. hirsutum* since the two species are incompatible).

4. **Mode of Reproduction.** In some cases, genes affecting the mode of reproduction have been transferred from wild relatives to the cultivated species. Self-incompatibility alleles from *B. Campestris* have been transferred to the self-compatible *B. napus* for the production of hybrid seed. Genes for apomixis have been transferred to maize (from *Tripsacum*) and sugarbeet (from wild *Beta* sp.). Cleistogamy and self-fertility traits of wild *Secale* sp. have been transferred to *Secale cereale.*

5. **Yield.** Contrary to the general belief, wild relatives of many crop species are excellent sources of the much needed 'yield genes'. An outstanding example of introgression of yield genes from a related wild species into a cultivated species is provided by the work of Frey and coworkers with oats (*Avena sativa*). *A. sativa* was crossed to *A. sterilis* ; the resulting F_1 was backcrossed four time to *A. savita.* In BC_4 and the subsequent selfed generations, selection for high yield resulted in the isolation of purelines which gave 25-30% more yield than the recurrent parent (*A. sativa* variety used as the parent in the hybridization programme). This yield increase is two-times the improvement obtained by intervarietal breeding programmes in U.S.A. during the period 1905 to 1960. Similarly, we have obtained equally encouraging results from interspecific hybridization in *Cicer.* The F_2 from the cross *C. arietinum* × *C. reticulatum* showed very large transgressive segregation for yield and several yield traits. Selections for increased 100-seed weight, pod number and seed yield per plant were effective ; F_6 progenies showing

upto 40% improvement in 100-seed weight and 30-40% increase in seed yield/plant over the *C. arietinum* parent have been isolated. Other crops where yield genes have been introgressed from the related wild species are : *Vigna*, *Zea*, *Arachis*, vanilla, tobacco and *Ribes*. Sugarcane is a special example as all the present-day varieties have two to three wild species in their parentage.

6. **Other Characters.** Wild relatives have provided genes for several other traits, *e.g.*, dark green colour and excellent leaf texture in lettuce ; short stature of plants in oil palm and wheat (from *Agropyron*) ; and bright red, thin-fleshed red peppers.

Many interspecific hybrids show genetic instability, possibly due to increase in the spontaneous mutation rate, *e.g.*, *N. tabacum* × *N. plumbaginifolia*, *Z. mays* × teosinte, some tomato hybrids etc. It is just possible that mutations occurring in such hybrids may be useful in crop improvement.

Transfer of Cytoplasm. Sometimes, it may be desirable to transfer cytoplasm of one species to another species. This is achieved by repeated backcrossing of the F_1 hybrid with the species to which the cytoplasm is to be transferred. The segregating progeny may be selected for fertility and for resemblance to the recurrent species. Five to six backcrosses may be sufficient in most of the cases. The most common and striking example of the transfer of cytoplasm is provided by the production of male sterile lines. Cytoplasmic male sterile lines of *N. tabacum* (tobacco) are produced by transferring the cytoplasm from *N. debneyi*, *N. megalosiphon* or *N. bigelovi* ; of *T. aestivum* (bread wheat) by transferring the cytoplasm from *T. timopheevii* or *Aegilops caudata* ; other crops where such transfers have been made are cotton, barley, potato. sunflower and ryegrass.

The foregoing transfers of chromosomes, chromosome segments, and of cytoplasm are examples of *introgression*. In introgression, few chromosomes or genes from a related species are transferred into the normal somatic chromosome complement of another species. Introgression is believed to have played a role in evolution of certain crop species, notably maize.

Utilization As New Varieties. In some cases, distant hybrids may be desirable enough to be used as new varieties. In such a case, the hybrid should not only be highly productive but must be easily produced at a commercial scale. There are, however, only a few examples of such an application. The hybrid cotton variety Varalakshmi is an interspecific hybrid and is very popular. It is a hybrid between *G. hirustum* and *G. barbadense* : it was developed at the University of Agricultural Sciences, Bangalore. It yields upto 50 quintals/hectare of long staple cotton which has a spinning count of upto 80. It is cultivated on an area of about 50,000 hectares. Subsequently, four more *G. hirsutum* × *G. barbadense* F_1 hybrids were released as commercial varieties ; these varieties are CBS 156,

Savitri, Jayalaxmi and K2HC. Sugarcane is a special case since all the present-day commercial varieties of sugarcane are complex interspecific hybrids involving *Saccharum officinarum* ($2n=80$), *S. spontaneum* ($2n=40$-218) and other species of *Saccharum*. The commercial varieties of sugarcane have a variable chromosome number ($2n=100$-125), 5-10 per cent of which are from the wild species. The commercial varieties are not only more resistant to diseases and pests in comparison to *S. officinarum*, but are also more productive. Bajra-napier grass hybrids yield twice as much or more fodder than the napier grass, the fodder is of superior quality as well. These napier-Bajra hybrids are becoming increasingly popular. The hybrid between *Brassica napus* and *B. campestris* has many desirable features and is easily produced. It is likely that this interspecific hybrid would be used as a hybrid variety in some parts of Europe.

There are some instances where new varieties have been isolated from the segregating generations of interspecific hybrids. A rice variety CO31 was developed from a cross between *Oryza sativa* (rice) and *O. perennis* (wild rice) using pedigree method of selection. Some rice varieties have been developed from crosses between the two subspecies of *O. sativa*, viz., *indica* and *japonica*. Adt. 27 was developed from the cross Norin 8 (*japonica*) × GEB24 (*indica*). Adt, 27 yields 5 tonnes/hectare of medium-fine rice of good cooking quality. In addition, it is early maturing and photoperiod insensitive.

Development of New Crop Species. Interspecific hybridization followed by chromosome doubling of the sterile hybrids produces allopolyploids that may serve as new crop species. More commonly, these synthetic allopolyploids serve as *bridging species* in crosses between two species with different levels of ploidy. For example, the hybrid between *N. tabacum* ($2n=48$) and *N. gultinosa* ($2n=24$) is sterile and cannot be used in gene transfers. But the amphidiploid *N. digluta* ($2n=72$) from the cross *N. tabacum* × *N. glutinosa* is reasonably fertile and produces partially fertile hybrids with *N. tabacum*. Thus the synthetic allopolyploid *N. digluta* serves as a bridging species between *N. glutinosa* and *N. tabacum*, and makes it possible to transfer genes and chromosomes from *N. glutinosa* to *N. tabacum*. Similar examples exist in wheat and cotton. Use of synthetic allopolyploids as bridging species is the most valuable and immediate application of allopolyploidy in crop improvement.

Development of new crop species by synthesizing allopolyploids is the most fancied goal and in a few cases promising results have been obtained. *Triticale* is now a promising crop species already in cultivation in some parts of the world. The history of triticales brings out two important points : *first*, a new (raw) amphidiploid/allopolyploid is unlikely to succeed as a new crop species and is likely to have several defects and undesirable features, and *second*, breeding and selection at the polyploid level would considerably improve the allopolyploid so that its defects may be corrected. There are indications that breeding of *Raphanobrassica*

may produce a useful crop species. There are a few other examples in other crops, *e.g.*, *Triticum-Aegilops* hybrids, *Festuca-Lolium* hybrids, hybrids in jute (*Corchorus*), *Rubus* etc.

LIMITATIONS OF DISTANT HYBRIDIZATION

An extensive use of distant hybridization in crop improvement has been limited by several problems summarised below.

1. *Incompatible Crosses.* Although many distant crosses can be made with the aid of various techniques, *several distant combinations cannot be crossed.* When a cross fails, it is generally difficult and time consuming to establish the exact cause of the failure. When the reason for the failure of a cross is not known, one can attempt to overcome the barrier only by trial and error. Distant hybridization is the most common, and perhaps the most useful, at the interspecific level (within the same genus), but several intergeneric crosses have also been of great promise, *e.g.*, *Triticale*, *Raphanobrassica* etc. Interfamily crosses are neither possible, and in all probability, nor profitable. A technique of somatic hybridization (Chapter 26) is being developed to produce incompatible distant crosses, but this technique is yet to be standardised and refined for a practical application.

2. *F_1 Sterility,* F_1 hybrids from distant crosses generally exhibit variable sterility ; rarely the F_1 is fully fertile, *e.g.*, F_1 from *Cicer arietinum* × *C. reticulatum.* Partial sterility of F_1 does not present a serious problem, and the F_1 may be either selfed or backcrossed to the cultivated species. Fully sterile F_1's are difficult to maintain, and in some cases doubling of the chromosome number of the F_1 may not be feasible or desirable. In any case, F_1 sterility confronts the breeder with a problem to overcome which he has to resort to specialised techniques demanding additional resources and time.

3. *Problems in Creating New Species.* Production of new crop species through allopolyploidy following distant hybridization suffers from several problems, *e.g.*, lower economic yields, poor agronomic characteristics, sterility etc. But these characters can be improved through breeding and selection as is evident from the history of triticales ; however, for this considerable effort, resources and time are required.

4. *Lack of Homology between Chromosomes of The Parental Species.* Transfer of oligogenes from related species is mainly limited by reduced pairing of the alien chromosome with that of the recipient species. Reduced recombination may be overcome by chromosome manipulation, but this is limited to polyploid species, and even there considerable cytogenetic analysis is required to make sure that only a small segment of the alien chromosome is transferred. In a vast majority of

cases, however, the gene transfer has to depend on spontaneous pairing and recombination. Elaborate techniques are being developed by E.R. Sears and C.J. Driscoll in wheat to promote crossing over between the alien chromosome segment carrying the desirable gene and the chromosome of the recipient species.

5. *Undesirable Linkages.* Linkage of the gene(s) under transfer with some undesirable gene(s) presents a major problem in using distant hybridization in crop improvement. In fact, undesirable linkages have prevented commercial exploitation of many alien gene transfers. In few cases, continued breeding has been able to break the undesirable linkages, but this demands considerable time, effort and resources.

6. In many distant hybridization programmes, transfer of recessive traits and of quantitative characters is not feasible. In fact, monogenic dominant characters are the most easily transferred, and such transfers constitute the bulk of interspecific gene transfers.

7. *Lack of Flowering in* F_1. F_1's from some interspecific hybrids fail to produce flowers. Hybrids between species belonging to the *Rhizomatosae* and those from the section *Arachis* of peanuts do not flower ; all the techniques to induce flowering in these hybrids have failed. Similarly, some interspecific hybrids in the genus *Glycine* fail to flower, but when they are grafted onto *G. max* they are readily induced to flower.

8. In distant hybridization programmes, it is important to use the best variety of a crop species as one of the parents. Transfer of genes from a related species into an agronomically poor type will necessitate further breeding efforts to use that gene. In many cases, the best varieties of a crop species cannot be crossed with the wild species; the wild relatives generally cross more easily with land races (old varieties) than with highly improved varieties.

9. *Dormancy.* In some cases, the F_1 seeds of an interspecific hybrid exhibit dormancy. For example, some interspecific hybrids in *Arachis* remain dormant for 5-10 years regardless of the treatments applied for inducing germination. Thus seed dormancy may render the use of some interspecific F_1 hybrids difficult, and in some cases, it may effectively prevent their utilization in crop improvement.

Some generalisations about the success of distant hybridization are as follows. (1) Polyploids are generally more likely to accept genes from other species than diploids because polyploids have a greater genetic buffering *i.e.*, the ability to tolerate genetic disturbances due to the presence of additional homologous or homoelogous genomes. (2) Cross-pollinated species are more likely to benefit from interspecific gene transfers due to their high heterozygosity. And

(3) Closely related species are the most likely to contribute useful genes to a crop species, chiefly due to the high homology between their chromosomes which facilitates gene transfers.

ACHIEVEMENTS

Distant hybridization has been most commonly used for the transfer of specific characters, *e.g.*, disease resistance to cultivated species. Many of the genes for rust resistance in wheat have been transferred from related wild species. Such examples are many in almost all the crop species, and this is where distant hybridization is likely to find a greater use in the future. Cytoplasm from related species has also been transferred to produce cytoplasmic male sterile lines, *e.g.*, in wheat (*T. aestivum*), tobacco (*N. tabacum*), cotton (*G. hirsutum*) etc. But these male sterility systems have not been commercially exploited due to one or the other problem in hybrid seed production, and in some cases the alien cytoplasm has undesirable side effects, *e.g.*, in tobacco.

A recent example of transfer of disease resistance from a related wild species to a cultivated species is found in bhindi.



Fig. 25.2. 'Parbhani Kranti', a bhindi (*Abelmoschos esculentus*) variety developed from the Cross *A. esculentus* × *A. manihot* (Courtesy, Dr. Y.S. Nerkar, Parbhani).

Parbhani Kranti bhindi is derived from the cross *Abelmoschusesculentus* cv. Pusa Sawani × *Abelmochus manihot* and is completely resistant to yellow vein mosaic virus. It yields 110-120 Q/ha in Kharif and 85-90 Q/ha in summer as compared to 105-110 Q/ha in Kharif and 75-80 Q/ha in summer of Pusa Sawani. Its fruits are dark green, slender and tender ; the variety is becoming popular throughout the country.

Distant hybridization has been employed for the development of new crop varieties, but such examples are limited to few crop species. Sugarcane appears to be a special case where improvement is based on distant hybridization. But this cannot be applicable to other crops, more particularly to diploid crops.

Distant hybridization coupled with chromosome doubling is a potential source of new crop species. This is illustrated by *Triticule*, which also clearly points out that extensive breeding can considerably improve inferior raw allopolyploids. Some other possibilities are *Raphanobrassica*, *Triticum-Agropyron*, *Triticum-Aegilops* and *Festuca-Lolium* hybrids etc.

SUMMARY

Hybridization between individuals from different species (belonging to the same genus or to different genera) is known as *distant hybridization*. Distant or wide crosses may fail due to failure of zygote formation, zygote development or development of hybrid seedlings. Zygote development may fail due to lethal genes, genetic imbalance, chromosome elimination, incompatible cytoplasm and endosperm abortion ; some of these mechanisms may be responsible for failure of the hybrid (F_1) seedling development. Distant hybrids show variable male sterility ranging from complete sterility to complete fertility. The sterility may be due to cytogenetic causes (reduced chromosome pairing, structural heterozygosity etc.), genetic factors or may be cytoplamically controlled. Genetic sterility is difficult to distinguish from that due to structural chromosome changes, particularly cryptic structural changes.

Segregation in distant crosses produces a very wide range of segregants, but this range represents only a portion of the possible segregants. Most of the segregants are weak and inferior to the parental species. The segregation pattern does not fit Mendelian ratios. Often distant hybrids have to be backcrossed (as female) to one of the parental species due to very high male sterility.

The most common use of distant hybridization in crop improvement is to transfer specific characteristics to cultivated species. Gene transfers are generally through recombination, but alien-addition and alien-substitution lines may be produced to permit the use of chromosome manipulation techniques for gene transfer. Chromosome manipulation is achieved by either promoting pairing of the alien chromosome with the chromosome of the recipient species, or by producing translocation with the aid of X-rays and gamma-rays. Distant hybridization has been used in transfer of cytoplasm, production of new varieties and development of new crop species. For producing new crop species, distant hybridization has to be coupled with chromosome doubling in the F_1 interspecific hybrids to produce amphidiploids.

QUESTIONS

1. Describe in brief the barriers in distant hybridization. How can these barriers be overcome ?
2. Discuss the salient features of segregation in distant hybrids.
3. What are alien-addition and alien-substitution lines ? Describe the procedure for their production with the help of suitable examples.

4. Discuss the various applications, achievements and limitations of distant hybridization in crop improvement.
5. Discuss the problems of sterility in wide crosses. Briefly explain the various mechanisms involved in sterility of distant hybrids.
6. Write short notes on the following : (i) genetic disharmony, (ii) endosperm abortion, (iii) chromosome elimination, (iv) alien-addition lines, (v) alien-substitution lines. (vi) bridging species, (vii) segregation in distant hybrids, and (viii) chromosome manipulation techniques.
7. Briefly describe the contributions of the following : (i) Thomas Fairchild, (ii) Karpechenko, (iii) Rimpau, (iv) Stebbins, (v) Oka, (vi) Sears, and (vii) Driscoll.

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CHAPTER 26

In Vitro Techniques in Plant Breeding

In vitro techniques relate to the cultivation of plant organs, tissues or cells in tesst tubes on artificial media. The conventional breeding methods are the most widely used for crop improvement. But in certain situations, these methods have to be supplemented with in vitro techniques either to increase their efficiency or to be able to achieve the objective which is not possible through the conventional methods. One example of each situation would illustrate the point. Production of purelines or inbreds involves six to seven generations of selfing. Production of haploids through distant crosses or through pollen culture, followed by chromosome doubling, reduces this time to two generations. This represents a saving of 4-6 years. The other example is the recovery of hybrids from distant crosses. Many distant crosses fail because of endosperm degeneration which blocks the development of the young embryo and may lead to its death. Removal of embryo at an early stage of development and its cultivation in vitro ensures a full development of the embryo and the recovery of hybrid plants from such crosses.

HISTORY OF IN VITRO TECHNIQUES

In vitro techniques were developed initially to demonstrate the totipotency of plant cells predicted by Haberlandt in 1902. *Totipotency* is the ability of a plant cell to perform all the functions of development which are characteristic of the zygote, *i.e*, its ability to develop into a complete plant. Efforts to demonstrate totipotency led to the development of techniques for cultivation of plant cells under defined conditions, that is, tissue culture. This was made possible by the brilliant contributions from R.J. Gautheret in France and from P.R. White in U.S.A. during the third and the fourth decades of the 20th century. *The first plant from a mature plant cell was regenerated in 1959 by Braun.* Most of the modern tissue culture media have been derived from the work of Skoog and coworkers during 1950s and 1960s. The first *embryo culture*, though crude, was done by Laibach in 1925 to recover hybrid progeny from an interspecific cross in *Linum*. Subsequently, contributions from several workers led to the refinement of this technique.

Haploid plants from pollen grains were first produced by Maheshwari and Guha in 1964 by culturing anthers of *Datura*. This marked the beginning of anther culture or pollen culture for

production of haploid plants. The technique has been further developed by many workers, more notably by J.P. Nitch, C. Nitch and coworkers. These workers have shown that isolated microspores of tobacco produce complete plants. *Plant protoplasts* are naked cells from which cell wall has been removed. In 1960, Cocking produced large quantities of protoplasts by using cell wall degrading enzymes. The techniques of protoplast production have now been considerably refined. It is now possible to regenerate whole plants from protoplasts and also to fuse protoplasts of different plant species. It may seem that the in vitro techniques are still in developing stages. But within a brief period, the techniques have made a great progress. From the sole objective of demonstrating totipotency of plant cells, the techniques now find application in both basic and applied researches in a number of fields of enquiry. Our concern here is to examine their present applications and future possibilities for improvement of crop plants.

THE TECHNIQUE OF IN VITRO CULTIVATION QF PLANT CELLS

The technique of in vitro cultivation of plant cells or organs is primarily devoted to solve two basic problems : *first*, to keep the plant cells and organs free from microbes, *i.e.*, bacteria and fungi, and *second*, to ensure the desired development in the cells and organs by providing suitable nutrient media and other environmental conditions. The first problem can be eliminated by using modern equipments and careful handling during various operations. The second problem remains the area of active research and is likely to do so for quite some time in the future. The technique of tissue, cell and organ culture is briefly outlined below. It may be pointed out that this account is essentially preliminary.

Surface Sterilization. The plant part to be cultured in vitro is known as *explant*. The explants must be surface sterilized to eliminate bacteria and fungi present on their surface. This is commonly achieved by treating them with 1-2 per cent solution of sodium or calcium hypochlorite or with 0.1% solution of mercuric chloride. The explant is then rinsed several times with sterilized distilled water to remove the disinfectant. Obviously, this and the subsequent handling of the explants or cultured cells and organs has to be done under aseptic conditions. *i.e.*, environment free from bacteria and fungal spores.

Nutrient Medium. The medium on which plant cells and organs are cultured is known as *nutrient medium*, *culture medium* or simply *medium*. The nutrient medium contains inorganic salts, trace elements, certain vitamins, a carbon source (generally sucrose) and, where needed, growth regulators, that is, auxins and/or kinins. There are many standard nutrient media available, but none of them is suitable for every purpose. Often, the experimenter has to make some modifications to develop a medium suitable for his own needs. Sometimes, complex organic supplements like coconut water, casein

hydrolysate and yeast extract are also used. The compositions of the two commonly used tissue culture media are listed in Tables 26.1 and 26.2.

Table 26.1. Composition of B5 medium for tissue culture of plants (Gamborg *et al.* 1968. Expl. Cell Res. 50: 151-158).

Compound	*Stock Solution (mg/1000 ml)*	*To make 1 litre*
$NaH_2PO_4.H_2O$		150 mg
KNO_3		2500 mg
$(NH_4)_2SO_4$		134 mg
$MgSO_4.7H_2O$		250 mg
Iron compound (330Fe)		28 mg
$CaCl_2.2H_2O$	150	1 ml
Micronutrients		
$MnSO_4.H_2O$	1000	
H_3BO_3	300	
$ZnSO_4.7H_2O$	200	1 ml
$Na_2MoO_4.2H_2O$	25	
$CuSO_4.5H_2O$	2.5	
$CoCl_2.6H_2O$	2.5	
KI	83	1 ml
Vitamins		
Nicotinic acid	10	
Thiamine HCl	100	10 ml
Pyridoxine HCl	10	
m-Inositol	1000	
Growth regulators		
2, 4-D (or any other auxin)	100	1 ml or as required
Kinetin (or any other kinin)	10	1 ml or as required
Sucrose		20 g
pH		5.5
Agar (for callus cultures)		6 g

The medium may be solidified by using agar (6g/1) or may be used as liquid. When liquid media are used, the culture flasks have to be constantly agitated or shaken on a gyratory shaker to facilitate aeration. The cells on an agar medium develop into an unorganised mass of cells known as *callus* ; consequently they are known as *callus cultures.* In the liquid medium, on the other hand, a suspension of free cells and small cell masses is obtained ; such a culture is known as *suspension culture.* The media are autoclaved to free them from microbes. Sterilized explants are then placed on nutrient media ; the operation is done under aseptic conditions.

Environmental Conditions. The organ and cell cultures are maintained under controlled environment, particularly temperature and light. The temperature may vary from 18-25°C depending upon species and the purpose of culture. Light is not essential for cell and tissue cultures, but it is beneficial for plantlet regeneration and for embryo and meristem cultures. The culture room or the incubator is kept as clean as possible to avoid contamination.

Table 26 2. Composition of MS tissue culture medium (Murashige and Skoog, Physiol. Plant. 1962. 15 : 473-497)

Compound	*Stock solution (mg/1000 ml)*	*Amount per litre*
NH_4NO_3		1650 mg
KNO_3		1950 mg
$MgSO_4\ 7H_2O$		370 mg
KH_2PO_4		170 mg
EDTA, Ferric salt		40 mg
$CaCl_2.\ 2H_2O$	15 g	2.9 ml
Micronutrients		
H_3BO_3	620	
$MNSO_4.4H_2O$	2230	
$ZnSO_4.7H_2O$	860	1 ml
$Na_2MoO_4.2H_2O$	25	
$CuSO_4.5H_2O$	2.5	
$CoCl_2.6H_2O$	2.5	
KI	83	1 ml
Vitamins		
Nicotinic acid	10	
Thiamine HCl	100	
Pyridoxine HCl	10	10 ml
m-Inositol	1000	
Growth regulators		
2, 4-D (or any other auxin)	100	1 ml (or as required)
Kinetin (or any other kinin)	10	1 ml (or as required)
Sucrose		30 g
pH		5.5
Agar (for callus culture)		6 g

Subculturing. After a period of time, it may be necessary to transfer organs and tissues to fresh media. This is particularly true of tissue and cell cultures where a portion of tissue is used to inoculate new culture tubes of flasks. This is known as *subculturing*. Plant cells and tissue cultures may be maintained indefinitely by serial subculturing.

Plant Regeneration and Transfer to Soil. The ultimate objective of the application of in vitro techniques to crop improvement is to obtain full plants and to transfer them successfully to soil. Production of various organs, *e.g.*, root, shoot etc., from cells and tissue cultures is known as *organ regeneration* or *organogenesis*. It is possible to regenerate plants from tissue cultures of a large number of species, including rice (*O. sativa*), maize (*Z. mays*), barley (*H. vulgare*), oats (*A. sativa*), sugarcane (*S. officinarum*), *Colocasia* (bunda and arvi), potato (*S. tuberosum*), pea (*P. sativum*), chickpea (*C. arietinum*) and alfalfa (*M. sativa*). But regeneration in many species, including most of the legumes, has not been obtained thus far. Regeneration capacity appears to be genetically controlled, and it may be possible to improve it by a suitable breeding programme. In alfalfa (*M. sativa*), two cycles of recurrent selection improved the regeneration capacity from 12 to 57 per cent.

Transfer of whole plants from test tubes to soil is relatively easy. The plants may be pretreated prior to their transfer to soil with different media designed to make them hardy. Generally, they are transferred into small pots and are covered with inverted beakers to prevent excess transpiration. After 3-4 days, the beakers are removed, but the pots are still kept in diffuse light for 5-10 days. The plants may now be transferred to sunlight. Seedling survival may vary from 50-100 per cent if care is taken.

CLASSIFICATION OF IN VITRO TECHNIQUES

The in vitro techniques are grouped into four categories on the basis of the plant part used as explant and the type of development in vitro. This classification is for convenience and is useful in discussions on the subject. The four groups of in vitro techniques are as follows : (1) embryo culture, (2) meristem culture, (3) pollen culture, and (4) tissue culture.

Embryo culture

In embryo culture, young embroys are removed from developing seeds and are placed on a suitable nutrient medium to obtain seedlings. The embryos generally do not complete development, but develop into seedlings. Sometimes, embroys from mature seeds may also be used for embroy culture, *e.g.*, in *Iris*, orchids etc. So far, it has not been possible to culture embroys before a certain stage of development, for example, before the globular stage in the case of barley. Elaborate media for embryo culture have been devised ; the tissue culture media may also be used for this purpose with appropriate modifications. Suitable techniques are available for embryo culture in many of the crop species, and may be easily developed for other species, if needed.

Applications of Embryo Culture. Embryo culture is being routinely used in some crop improvement programmes to obtain interspecific hybrids. It has some other specific uses as well, for example, propagation of orchids, overcoming dormancy etc. The various applications of embryo culture are described below.

Recovery of Distant Hybrids. Distant crosses may fail due to one or more of several reasons, *e.g.*, inability of pollen to germinate, failure of the pollen tubes to grow or, perhaps more commonly, due to the degeneration of endosperm. When embryo fails to develop due to endosperm degeneration, embryo culture is used to recover hybrid plants. Embryo culture has been widely used for this purpose. Some recent examples are the recovery of hybrids from *Hordeum vulgare* × *Secale cereale*, *Triticum aestivum* × *Agropyron repens*, *H. vulgare* × *Triticum* species including *T. aestivum* etc. Two examples of such an application of embryo culture will illustrate the point. In case of *Triticale*, rare combinations between *Triticum* and *Secale* develop viable seeds. But most of the tetraploid and hexaploid wheats carry two dominant genes, Kr_1 and Kr_2, which prevent seed development in crosses with *Secale*. The majority of hybrid seeds are small,

poorly developed and show very poor germination. Further, seeds are obtained from only 5-10 per cent of the florets pollinated. The recovery of hybrid seedlings is much greater (50-70 per cent) when embroys from 10-14-days old caryopses are removed and cultured on a suitable medium.

Hybrid seeds from *T. aestivum* × *H. vulgare* are not obtained, but hybrid seedlings have been obtained by embryo culture. Hybrid embryos from 9-12 days old caryopses were removed and placed on 14-18 days old barley endosperm cultured on a nutrient medium. As many as 10 hybrid embryos may be placed on a single endosperm. A high proportion of hybrid embroys developed into seedlings when they were thus cultured.

Recovery of Haploid Plants from Interspecific Crosses. When *H. vulgare* or *T. aestivum* (used as male) is crossed with *Hordeum bulbosum* (used as female), the chromosome complement of *H. bulbosum* is eliminated from the developing embryo. Most of the seedlings obtained from such crosses are haploid, having only one set of chromosomes from *H. vulgare* or *T. aestivum* parent. There is some seeds set in *H. bulbosum* × *T. aestivum* crosses, but caryopses tend to degenerate after 10 days in the case of *H. bulbosum* × *H. vulgare* crosses. Embryo culture of 8-10-days old embryos enables the recovery of a large number of haploid seedlings in both the cases.

Propagation of Orchids. Orchids are difficult to propagate since their seeds lack any stored food and the embryo is virtually naked. In many orchids, embryo development is incomplete at the time the seeds mature. Young or mature orchids embryos are removed from seeds and placed on suitable nutrient media. The embryos develop into seedlings either directly or through callus formation.

Shortening The Breeding Cycle. Seeds may take 10-20 days to mature after the embryo is sufficiently developed to permit successful culture in vitro. The embryos do not complete their development in vitro, but develop directly into seedlings. Thus the next generation may be grown one or two weeks earlier by embryo culture than from seeds. But this has not become a practical feasibility because of two reasons : *first*, embryo culture is an exacting and time consuming technique and *second*, excellent green house facilities are a prerequisite for such an application of the technique.

Overcoming Dormancy. In some fruit trees, embryos require a period of after-ripening before germination. *Iris* seeds may take two to several years to germinate. This period of dormancy can be effectively eliminated by embryo culture. Other examples are, *Prunus*, *Taxus* etc.

Meristem Culture

The cultivation of apical meristems, particularly of shoot apical meristem, is known as meristem culture. Meristem culture involves the development of an already existing shoot apical meristem and the regeneration of adventitious roots. It does not involve the

regeneration of a new shoot apical meristem. Meristem cultures have been extensively used for quick vegetative propagation of a large number of plant species. Ordinarily, it is not necessary to excise or isolate the apical meristem for meristem culture. Usually, 2-3 mm shoot apices containing the shoot apical meristem alongwith several leaf primordia are used. When the objective is vegetative propagation, the size of shoot tip used for culture is not important. But when the objective is to free the stock from a virus, it is essential that the apical meristem should be excised alongwith the minimum of the surrounding tissue. This is necessary in order to get rid of the virus particles that may be present in the surrounding tissues. The shoot tip may be cut into fine pieces to obtain more than one plant from each shoot-tip. Axillary buds may also be used for meristem culture. The standard tissue culture media are suitable for this purpose with some modifications, if necessary.

Applications of Meristem Culture. Meristem culture has many useful applications in crop improvement : clonal propagation, recovery of virus-free stocks, germplasm exchange and germplasm conservation.

Vegetative Propagation, Meristem culture has been extensively used for vegetative propagation of many crops and fruit trees. Meristem culture is used for obtaining clones, hence it has been termed as *mericloning, i.e.,* cloning through meristem. A single meristem usually produces many plantlets which may again be used for meristem culture. Mericloning is useful in clonal multiplication of vegetatively propagated crops, *e.g.,* ginger (*Zingiber* sp.), turmeric (*C. domestica*) etc , and of fruit and timber trees, and in the maintenance and quick multiplication of some breeding materials.

Recovery of Virus-Free Stocks. Seeds from virus-infected plants generally do not contain the virus. Therefore, sexual progeny are usually virus-free, except for new infections. But in case of asexually reproducing crops, virus infections spread rapidly. This is because vegetative propagules from virus-infected plants contain virus particles. But the shoot apical meristems and some young tissues surrounding them are generally free from viruses. Meristem culture is, therefore, useful in recovering virus-free plants from virus-infected plants or clones.

Many valuable clones in potato (*S. tuberosum*), sugarcane (*S. officinarum*) and other clonal crops have been lost due to viral infections. Some valuable clones in potato, sugarcane etc. have been freed from virus infections through meristem culture. Care must be taken to remove the apical meristem with as little surrounding tissue as possible to make sure that virus particles are not present in the explant. This application of meristem culture is of great value, particularly in the maintenance of breeding materials and germplasm which are invaluable for any breeding programme.

Germplasm Exchange of Clonal Crops And Fruits. The commonly used propagules in most of the clonal crops, particularly in the case

of those that do not produce seeds, are rather bulky. Further, there is the necessity of careful quarantine. Seedlings in test tubes obtained from apical meristems would prove extremely useful in germplasm exchange of such crops. Such seedlings would be free from pathogens and insects, and would be easier to handle. This application is likely to become considerably important in the future.

Germplasm Conservation. Germplasm conservation in clonal crops, particularly in case of root and tuber crops, and in trees presents many problems. Roots and tubers lose viability rapidly and their cold storage requires large space and is expensive. Freeze preservation of meristems and cells in liquid nitrogen at −196°C has been suggested for the long-term preservation of their germplasm. This technique is still in the developing stages ; we shall discuss more about it later in this chapter.

At present, meristem culture is commercially used for quick clonal multiplication of many valuable crops. In some cases, it has been used to obtain virus-free clones. The other two objectives, *viz.*, germplasm exchange and conservation, are yet to be used on a substantial scale.

Anther or Pollen Culture

Haploid plants may be obtained from pollen grains by placing anthers or isolated pollen grains on a suitable culture medium. The most suitable stage of the pollen grains for this purpose appears to be the binucleate stage, that is, after the first mitotic division of the pollen nucleus. The generative nucleus, *i.e.*, the nucleus that would have produced the sperms, degenerates. The vegetative nucleus divides mitotically to produce either an embryoid or a callus mass. Embryoids are directly produced from pollen grains in *Datura* and tobacco. In such cases, the plants obtained from the embryoids are generally haploid, but some polyploid plants are also produced. In many species like rice (*O. sativa*), barley (*H. vulgare*), wheat, triticales etc., pollen grains produce callus from which plantlets may be regenerated under suitable culture conditions. In these cases, the ploidy level of plants varies considerably more than in those where embryoids are produced. Haploid plantlets have been regenerated from pollen grains of the following crop species : potato (*S. tuberosum*), barley, wheat (*Triticum* sp.), rice, *Brassica campestris*, *Triticale*, many members of Solanaceae and some vegetables.

Applications of Anther Culture. Anther/pollen cultures are useful in obtaining haploid plants. Haploid plants are of considerable value in genetic studies and in plant breeding. Haploids are of considerable value in obtaining homozygous diploids in a short period of time. There is some evidence that selection based on haploids may be 6-8 times as efficient as that based on zygotic diploids. Haploids obtained from pollen grains are being used for the improvement of tobacco where disease resistant and promising lines have been developed through this technique. In China, the technique is being used in the improvement of rice and wheat. A promising variety of

rice, Tanfeng (meaning haploid derived, high yielding No. 1) has been released for commercial cultivation. In many crops, the application of this technique is not yet practical because large numbers of haploid plants are not easily obtained. Therefore, a wider application of pollen culture in crop improvement depends primarily upon the development of techniques for quick production of large numbers of haploid plants.

There is considerable evidence that haploid plants regenerated from callus cultures show genetic variation, that is *gametoclonal variation*. For example, gemetoclonal variation for heading date, plant height etc. has been reported in rice. Such a variation may be subjected to selection at the haploid level, and the chromosome number of the selected plants may be doubled to obtain homozygous plants. Gametoclonal variation appears to be more desirable than somoclonal variation because (1) the mutant characteristic is expressed in the R_0 plants, *i.e.*, plants regenerated from the cells in vitro, and (2) cells having detrimental mutations may be expected to regenerate much less frequently in the case of haploid than in diploid cells. However, regeneration of haploid plants is feasible in fewer plant species than those from somatic cells.

Tissue and Cell Cultures

Application of plant tissue and cell culture in crop improvement depends solely on the availability of techniques for regeneration of whole plants from them. Both callus and suspension cultures of many species regenerate whole plants. In some cases, there is production of somatic embryos, *e.g.*, carrot, sandalwood tree, *Dioscorea* (yam) etc., but this is limited to a few species. In many other species, however, shoot buds differentiate from the cell mass followed by differentiation of roots, *e.g.*, tobacco, rice, wheat, barley, coffee (*Coffea arabica*) etc. Regenerated plants often show variation in ploidy level due to chromosome instability of plant tissue cultures. Generally, the chromosome instability of tissue cultures increases with the duration of in vitro culture, while their regenerating capacity decreases. The tissue culture technique has several applications in crop improvement some of which are being exploited at present.

Applications of Tissue and Cell Cultures. The various applications of plant tissue and cell cultures are briefly discussed below.

Clonal Propagation. Tissue culture is well suited for quick vegetative propagation of plant species. This is particularly true for those cases where regeneration of plants from callus and suspension cultures is relatively easy. It is used for asexual propagation in many species including some fruit and timber trees. In some cases, it has been used for obtaining disease-free and virus-free plants. Quick multiplication of plant cells in vitro often 'cures' them of viral infections. Therefore, plants regenerated from them are generally virus-free. The major difficulty in the use of this technique in clonal multiplication is the occurrence of genetic variation among the regenerated

plants. This variation may arise due to chromosomal aberrations or gene mutations. This problem can be reduced to a large extent by using young tissue cultures, preferably during the first few subcultures.

Mutant Isolation. Biochemical mutants are far more easily isolated from cell cultures than from whole plant populations. This is because a large number of cells, say $10^6 - 10^9$, can be easily and effectively screened for biochemical mutant cells. Screening of as many plants would be very difficult, ordinarily impossible. Biochemical mutants could be selected for disease resistance, improvement of nutritional quality, adaptation of plants to stress conditions, *e.g.*, saline soils, and to increase the biosynthesis of plant products used for medicinal or industrial purposes.

1. *Somaclonal Variation*. Plants regenerated from tissue and cell cultures show heritable variation for both qualitative and quantitative traits ; such a variation is known as *somaclonal variation*. Somaclonal variation has been described in sugarcane, potato, tomato etc. Some variants are obtained in homozygous condition in the plants regenerated from the cells in vitro (R_0 generation), but most variants are recovered in the selfed progeny of the tissue culture-regenerated plants (R_1 generation). Somaclonal variation most likely arises as a result of chromosome structural changes, *e.g.*, small deletions and duplications, gene mutations, plasma gene mutations, mitotic crossing over and, possibly, transposons. Most of the variants in tomato were shown to be recessive gene mutations, and homozygous mutants recovered in the R_1 generation were most likely produced by mitotic crossing over in the cells before regeneration of the shoot-tip. Some plasma gene mutations have also been recovered. Most of the mutations recovered in tomato were identical with the already known spontaneous or induced mutations, but some mutations were entirely new. Somaclonal variation may be profitably utilized in crop improvement since it reduces the time required for releasing the new variety by at least two years as compared to mutation breeding and by three years in comparison to backcross method of gene transfer.

2. *Amino Acid Analogue Resistant Mutants*. Cereal grains are deficient in lysine ; maize (*Z. mays*) is also deficient in tryptophan, while wheat (*T. aestivum*) and rice (*O. sativa*) are deficient in threonine. Pulses are deficient in methionine and tryptophan. The concentration of amino acids in the free amino acid pool of cells is primarily controlled by feedback inhibition of the enzymes of ammo acid biosynthesis. Amino acid analogue-resistant cells may be expected to show a relatively higher concentration of that particular amino acid. For example, carrot (*D. carota*) and tobacco (*N. tabacum*) cell lines resistant to tryptophan analogue 5-methyl tryptophan show a 10-27-fold increase in the level of trytophan. Similarly, rice cells resistant to lysine analogue 5-(β-aminoethyl)-cysteine show much higher levels of lysine. This technique may prove useful in the

development of crop varieties with a better balanced amino acid content. But this approach seems to have reached a dead end as the seeds produced by the mutant lines do not show higher concentrations of the particular amino acids present in the plants. But the approach may yet find a use in vegetable crops where the vegetative parts of plants are consumed.

3. *Disease Resistant Mutants.* Many pathogenic bacteria produce toxins that are toxic to plant cells. Plant cell cultures may be exposed to lethal concentrations of these toxins and resistant clones isolated. Plants regenerated from these resistant clones would be resistant to the disease producing pathogen. This technique should be applicable to all the pathogens which produce the disease through the action of a toxin. An example of such an application is in the case of wildfire disease of tobacco (*N. tabacum*) produced by *Pseudomonos tabaci.* Tobacco cells resistant to methionine sulfoximine, which is similar to the toxin produced by the pathogen, were isolated. Plants regenerated from these clones were resistant to wildfire disease, although to a somewhat lesser degree. Similarly, maize (*Z. mays*) lines having the Texas male sterile cytoplasm are susceptible to Southern leaf blight caused by *Helminthosporium maydis*, which produces a toxin that binds with mitochondria. Maize cells resistant to this toxin have been selected, and plants regenerated from them were resistant to leaf blight caused by *H. maydis.* These examples show the potential of the technique. But much work is needed before the technique would be of general applicability. Further, the technique can be applied to those cases only where the disease is the result of a toxin produced by the pathogen. But many of the pathogens do not seem to produce a toxin, or the toxin does not appear to be the primary cause of the disease.

4. *Stress Resistant And Other Mutants.* Plant cells resistant to 4-5 times the normally toxic salt (NaCl) concentration have been isolated. In many of the cases, the plants regenerated from them were also tolerant to saline conditions. Damage due to water-logging presumaly occurs due to alcohol accumulation in roots. It may be expected that mutant cells lacking alcohol dehydrogenase would accumulate very little alcohol and, therefore, would be water-logging tolerant. Attempts to isolate such cells are being made. Similarly, attempts are being made to isolate clones that would produce more substances of medicinal or industrial value.

Somatic Hybridization. Protoplasts can be isolated from almost every plant species and cultured to produce callus. Protoplasts of two different species may be fused with the help of polyethylene glycol. In some cases, hybrid plants have been obtained after fusion of protoplasts, *e.g.*, from fusion of *Nicotiana glauca* protoplasts with those of *N. langsdorfii*, two mutant lines of *N. tabacum* (tobacco); and two sexually compatible species of *Petunia* (*P. parodii* and *P. hybrida*). It was once suggested that somatic hybrids would be of great value in crop improvement. But the experience with sexually

produced interspecific distant hybrids (Chapter 25) is not very encouraging ; *Triticale* is the exception to the general picture. Further, at present the techniques for selection and multiplication of somatic hybrid cells and the regeneration of hybrid plants from them is limited to a few special cases. It is doubtful that somatic hybridization would be of any practical value in crop improvement in the near future.

Genetic Transformation. There is some evidence that gene transfer may be achieved by feeding cells with DNA in case of eukaryotes, such as, *Drosophila, Neurospora,* cultured mammalian cells and in some plants. Genetic changes may be brought about by DNA or by radiation-killed pollen grains. The exact nature of the molecular events associated with these genetic changes is not known. This raises the possibility of genetic modification of plant cells with the help of both homologous (from the same species) and heterologous (from a different species) DNA. It is also proposed that DNA plant viruses, such as cauliflower (*B. oleracea*) mosaic virus and potato leaf roll virus, plasmids (*e.g.*, Ti plasmid of *Agrobacterium*) and transposons, may be used as the carriers of genes for genetic modification of plant cells. The techniques are yet to be developed for general applicability, and their usefulness in crop improvement is yet uncertain, although some promising gene transfers have already been made using the Ti plasmid as vector.

Organelle Transfer. In some cases, it may be desirable to transfer only organelles or the cytoplasm into a new genetic background. This may be achieved through the use of plant protoplasts. Chloroplasts have been transferred, and other organelles including nucleus may be transferred. The technique is yet to be refined for practical application.

Germplasm Coservation. It has been suggested that tissue cultures may be frozen and stored in liquid nitrogen at −196°C for long-term storage of germplasm. This would be of great value in the conservation of germplasm of those crops which normally do not produce seeds, *e.g.*, root and tuber crops, or where it may not be desirable to store seeds. For freeze-preservation, the cells are cooled at a slow rate and are then transferred to liquid nitrogen for storage. Thawing of the cells must be very rapid for increased survival. A cryoprotectant, such as dimethylsulfoxide (DMSO), is used to protect the cells from injury due to freezing and thawing. The technique of freeze-preservation, *i.e., cryobiology*, of plant cells is still in the developing stages. In future, it may assume considerable importance in germplasm conservation, particularly of those species that do not produce seeds.

ACHIEVEMENTS AND FUTURE PROSPECTS

Embryo culture is routinely used for the recovery of hybrid plants from distant crosses. This is particularly useful where embryos fail to develop due to endosperm degeneration. It is being used extensively in the extraction of haploid barley (*H. vulgare*) from

H. bulbosum × *H. vulgare* crosses. Barley breeding programmes based on haploids thus derived have been initiated in several countries and many varieties have resulted from these programmes. Embryo culture is also routine in orchid propagation and in breeding of those species that show dormancy, *e.g.*, *Iris.*

Meristem culture and tissue culture are being widely used in vegetative propagation of many crop species. They are also used for obtaining virus-free plants from virus-infected clones. These techniques hold considerable promise in germplasm conservation and exchange. Tissue culture may prove to be an important tool in the isolation of biochemical mutants with improved nutritional quality, resistance to diseases, tolerance to stress conditions etc. Somaclonal variation has been exploited develop some sugarcane varities in India, *e.g.*, Co 85001, Co 85003, Co 85006, Co 85007, Co 85008, Co 85011 and Co 85015. Genetic changes may also be brought about by the use of DNA, DNA viruses and transposons. But these are only potential applications. Much basic work is needed before these techniques would become of general practical use.

Haploids are being produced in tobacco, wheat and rice through pollen culture. These are being used for the development of disease resistant and superior diploid lines. In China, about two dozen rice varieties and some wheat varieties have been developed through this technique. Further work is likely to enable the application of this technique in many other crop species.

The chief problem of tissue and pollen culture is the difficulty in obtaining regeneration of plants in large numbers. Development of suitable media and selection for genotypes with higher regeneration capacity may improve plant regeneration in many species. The future prospects of plant tissue culture and pollen culture would largely depend upon the availability of techniques for easy regeneration of large numbers of plants.

The in vitro technique is highly sophisticated and requires both environmental and biological (contamination) control. It is likely that it may not ever be of general application in crop improvement. Most likely, a few centres may specialise in these techniques and would generate materials for the use of plant breeders in crop improvement programmes.

SUMMARY

The in vitro techniques consist of maintenance of plant organs and cells on defined media in test tubes. Tissue culture began with the idea to demonstrate totipotency of plant cells. But now it has many applications. There are mainly four types of in vitro techniques : embryo culture, meristem culture, pollen culture and tissue culture. *Embryo culture* involves removal of young embryos from developing seeds and growing them on suitable culture media. Embryo culture is useful in recovery of hybrid plants from distant crosses, recovery of haploid plants from certain crosses, propagation of orchids, shortening of breeding cycle and overcoming dormancy. In *meristem culture*, shoot apical meristem alongwith some surrounding tissue is grown in vitro. It is used for clonal propagation and recovery of virus-free plants and in potentially useful in germplasm exchange and long-term storage of germplasm

through freeze-preservation. *Pollen culture* is culture of anthers or isolated pollen grains in vitro. The vegetative nucleus of pollen grains divides to produce embryoids or callus masses. Pollen culture is used to obtain haploid plants which are being used in tobacco and rice improvement. *Tissue culture* consists of growing plant cells as relatively unorganised masses of cells on an agar medium (*callus culture*) or as a suspension of free cells and small cell masses in a liquid medium (*suspension culture*). Tissue culture is used for vegetative multiplication of many species and, in some cases, for recovery of virus-free plants. It has potential applications in production of somatic hybrids, organelle and cytoplasm transfer, genetic transformation and germplasm storage through freeze-preservation.

In vitro techniques are being used in certain special situations. But they are not of more general application for two principal reasons : *first*, regeneration of large number of plants is not possible in most of the species and *second*, the technique requires much expertise and a well equipped laboratory. A *third* reason is that the techniques are still in the developing stages and it would be some time before they become standardised and generally applicable.

QUESTIONS

1. Discuss the possibilities offered by in vitro techniques in crop improvement.
3. List the various types of in vitro techniques. Describe in brief any two of them and discuss their applications and limitations.
3. Define the following : totipotency. organogenesis. explant, callus, cryobiology, mericloning, somatic hybridization, tissue culture.
4. Write short notes on the following : (i) pollen culture, (ii) tissue culture, (iii) meristem culture, (iv) embryo culture, and (v) regeneration of plants from cells cultivated in vitro.
5. Briefly outline the procedure and technique of in vitro culture of plant cells and organs.

Suggested Further Readings

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CHAPTER 27

Release of New Varieties

The ultimate aim of any breeding programme is to develop varieties superior to the existing ones in yielding ability, disease and insect resistance and other characteristics. The various methods of breeding are designed to help the breeder in developing such superior strains. For commercial utilization these strains have to be released as varieties by either the Central or a State Variety Release Committee. The release of a strain for use as a variety is based on conclusive demonstration of its superiority over the best existing varieties (included as checks in the evaluation trials) in yielding ability or in some other feature of economic importance, *e.g.*, disease resistance, drought tolerance, salt tolerance, etc. For this reason, the strains are extensively evaluated for their performance, disease resistance, quality etc. in multilocation trials. The multilocation trials are conducted under the All India Coordinated Crop Improvement Projects, which play a key role in testing, identification and release of new varieties. But before the new strains are included in multilocation tests, the breeder should evaluate their performance at his own station to make sure that the new strain is superior to the existing varieties.

The various activities and operations in the release of a strain as a variety may be grouped into the following three classes for convenience in description : (1) evaluation, (2) identification and (3) release and notification. Following the notification of a variety, its seed multiplication is undertaken.

Evaluation

Evaluation of a strain for release as a variety consists of various trials and tests to determine its superiority over the best existing variety in yield and other agronomic traits, and its suitability for consumption. In general, there are seven different types of trials/tests : (1) station trial, (2) multilocation trials, (3) national trials, (4) adoptive trials, (5) minikit trials, (6) disease and insect tests, and (7) quality tests. In all these trials, the best existing variety(ies) is included as a check for comparison ; in general, more than one (often three) variety is included as check.

Station Trial. Station trial is conducted by the breeder who has developed the new strain(s). Such a trial is often referred to as preliminary yield trial and may be conducted for one or more years. The objective of station trial is to make sure that the new strains

developed by a breeder are superior in performance (at that location) to the best available variety for the region before they are included in the trials of the concerned All India Coordinated Crop Improvement Project. This is of great importance because in the trials of coordinated projects these strains would not only have to compete with the best existing variety, but also with the new strains developed by other breeders. Therefore, to avoid disappointment and rejection in the early stages of testing, the breeder should himself make sure of the superiority of the strains he has isolated. In addition, multilocation trials require considerable human and financial resources, which are but limited in a developing country like India. It is highly desirable to utilize these scarce resources for the evaluation of only promising new strains and not to waste them in the testing of inferior entries of little agronomic worth.

In station trials of wheat, the plot size is generally 5 m × 1.8 m with a spacing of 23 cm between rows (the same spacing is used in all the yield trials of wheat). The seed rate is at the commercial planting rate (100 kg/ha). Plant to plant spacing is not maintained, but it is important that the stand should be uniform. The number of replications may vary, but should not be less than 3 to 4. The best existing varieties are used as checks. Disease reaction and quality of the new strains are also evaluated. The station trial may be repeated once to make sure that the new strains are superior to the existing varieties.

The data from station trials are not required for the inclusion of a strain in the multilocation trials ; wheat seems to be an exception where station trial data are proposed to be considered.

Multilocation Trials. These trials are conducted under the respective All India Coordinated Crop Improvement Projects. The objective of these trials is to evaluate the performance of newly developed strains at several locations distributed over a region. Since the soil and climatic (agroclimatic) conditions show a large variation from one region of the country to the other, the country has been divided into several agroclimatic zones ; each zone consists of areas having similar agroclimatic conditions. The number of zones and the zoning pattern vary considerably from one crop to the other. The pattern of zones for a crops is based on several considerations, including the distribution pattern of the crop and the area under it in the various regions. The number of zones for a crop varies from one in the case of jowar (sorghum) and bajra, through two in the cases of rajma (North-Eastern Plains Zone and Central Zone) and safflower (traditional and nontraditional) to nine in the case of wheat and gram (Table 27.1)

The evaluation procedures of wheat are considerably refined, and they have served as a model for those of several other crops. The description of the nine wheat evaluation zones is summarised in Table 27.2. In the subsequent sections, the evaluation procedure of wheat is described in some detail.

Table 27.1. Description of the nine agroclimatic zones for evaluation of wheat varieties.

Zone	*Areas*
Northern Hills Zone (NHZ)	Hills of Uttar Pradesh, Himachal Pradesh, and Jammu and Kashmir.
Northern Plains Zones (NPZ)	Punjab, East Haryana, West U.P., Delhi, Ganganagar, Alwar, Bharatpur (Rajasthan), Gwalior, Bhind, Morena (M.P.), Uno and Panota Valley (H.P.); Jammu (J. & K.)
North-Western Plains Zone (NWPZ)	Rajasthan (except Kota and Udaipur), Western parts of Haryana, parts of Gujarat.
North-Eastern Plains Zone (NEPZ)	Eastern U.P., Bihar (except Chhota Nagpur)
Far Eastern Zone (FEZ)	W. Bengal, Bihar (Chhota Nagpur), Assam, Sikkim, Arunachal Pradesh, Tripura, Manipur, Mizoram, Nagaland, Meghalaya.
Central Zone (CZ)	Kota and Udaipur (Rajasthan), Jhansi (U.P.), parts of Gujarat, M.P. (except Eastern and South-Eastern parts).
South-Eastern Zone (SEZ)	Orissa, South and South Eastern M.P., Some parts of Maharashtra, A.P. (East Coast region).
Peninsular Zone (PZ)	Karnataka, West A.P., plains of Tamil Nadu, remaining parts of Maharashtra.
Southern Hills Zone (SHZ)	Hills of Tamil Nadu.

Table 27.2. A summary of the zoning patterns and the evaluation procedures used for testing of new strains in different crops.

Crop	*Zones**	*Evaluation procedure***	*Condition of testing*
		Cereals	
Wheat	9 (NHZ, NPZ, NWPZ, NFPZ, EPZ, FEZ, CZ, PZ, SHZ)	IET, 1 Yr URT, 2 Yrs AT, 1 Yr	1. Timely sown (15-25 Nov.), Irrigated 2. Late sown (15-25 Dec.), irrigated 3. Rainfed (around 31 Oct.)
Rice	8 (NEPZ, NHZ, NPZ, CZ, EZ, FEZ, SWZ, SZ)	PVT, 2 Yrs UVT, 2 Yrs AT, 1 Yr	1. Rainfed (direct-seeded) 2. Transplanted 3. Direct seeded 4. Deep water 5. Saline/alkaline soil 6. Aromatic, slender grain
Barley	5 (NEPZ, NWPZ, CZ, Diaraland Zone, Hill Zone)	IET, 1 Yr URT, 2 Yr AT, 1Yr	**A. Hulled** 1. Timely sown, irrigated 2. Late sown, irrigated 3. Timely sown, rainfed **B. Hull-less** 4. Timely sown irrigated 5. Late sown, irrigated 6. Timely sown, rainfed **C. Winter barley (in Hill Zone only)**

Famine Commission (1944), and Food Grains Policy Committee (1944).

Quality seeds of vegetables were being imported from countries like Australia, U.K., U.S.A., Germany etc. upto 1939. In 1945, some private seed companies, *e.g.*, Sutton's, Pocha etc., began quality seed production of temperature vegetables in Quetta and Kashmir Valley ; they formed an All India Seed Producers' Association in 1946. Several private seed companies (see later) were formed during 1950s and 1960s ; during 1970s an estimated 200 private companies were involved in the production and marketing of quality seeds of various crops.

Both crop improvement and quality seed production received impetus after the independence. In 1952, the Indian Council of Agricultural Research appointed a Standing Experts Committee on Seeds which formulated a programme for strengthening the seed production and distribution systems. As a consequence, the Central Government provided financial assistance to the states for this purpose, and in 1956-57 the 'State Seed Farm Project' was initiated. Under this programme, states began production foundation seed on their State Seed Farms, but this programme was confined to cereals only. In each development block a 10 ha seed farm was established during the first five year plan ; these farms were 4,328 in number, covered an area of 43,280 ha and involved an expenditure of 18 crore rupees.

In 1959, the Agricultural Production Team, headed by Dr. Johnson recommended that uniform standards of seed certification and seed laws should be brought into force and that each state should establish seed testing laboratories. The Planning Commission appointed a Seed Multiplication Team to review the various aspects of seed programmes ; this team made several valuable recommendations. Similarly, the ICAR set up a committee in 1960 in order to suggest ways for developing a strong seed production programme; this committee suggested the establishment of central and state agencies for the production of foundation seed, the establishment of independent seed certification agency, the enactment of a national seeds act and the creation of agencies for its enforcement, and for stimulating the development of the private seed industry. Based on these recommendations, the National Seeds Corporation (NSC) was established in 1963, and the Indian Seed Act was enacted in 1966. The Government of India set up a Seeds Review Team in 1968 ; the team toured several foreign countries and made some far-reaching recommendations. One of the recommendations was that Agricultural Universities should also be involved in foundation seed production.

The seed industry developed fairly rapidly with the establishment of NSC. Both the quantity of quality seeds produced and the number of crops covered has rapidly expanded. For example, the total quantity of quality seed produced in 1966-67 was only 36,080 quintals as compared to 48.46 lakh quintals in 1984-85 (Table 28.1).

different types of seeds produced. The different types of seed denote different stages in multiplication of a variety, and different levels of genetic and physical purity as well.

HISTORICAL

The procedures for seed production and processing and the standards for seed certification developed slowly with the realization of the importance of quality seed in agriculture. Most likely, seed certification began in Sweden during the last quarter of the nineteenth century. In 1886, the Swedish Seed Association was formed ; it undertook the production and distribution of quality seeds of improved varieties of mainly forage crops. Towards the end of the 19th century, Dr. E. Helvo established a seed testing laboratory in Denmark in order to conduct seed tests for certification. In 1917, Dr. J.W. Robertson, a Canadian scientist, proposed the production of foundation seed, which is now a crucial stage in quality seed production.

In 1919, the International Crop Improvement Association (renamed as Association of Seed Certification Agencies in 1969) was formed. This Association has been crucial in the development of the procedures and the standards for quality seed production and seed certification. In 1946, the Association clearly defined the four classes (breeder seed, foundation seed, registered seed and certified seed ; see later) of quality seeds in relation to forage crops. Later, in 1968, the Association proposed that the same four seed classes be adopted for grain crops as well. The procedures and the minimum quality standards for seed production, defined and published by the association in 1946, have been used, with appropriate modifications by U.S.A., Canada and many other countries, including India.

DEVELOPMENT OF SEED INDUSTRY IN INDIA

In India, new improved varieties of some important crops, notably wheat, were developed during the first two decades of the twentieth century. Efforts were made by the State Departments of Agriculture and some other agencies. *e.g.*, some princely states, to disseminate the seeds of these improved varieties among the farmers. For example, the State Department of Agriculture, U.P., produced and distributed more than 15,000 quintals wheat seed. A limited facility for seed test was available at Kanpur. During 1920's the Government of U.P. initiated a project for establishing seed godowns in every subdivision (*tehsil*). Clearly, the then government was amply aware of the importance of quality seeds and efforts were initiated for their production and distribution.

In 1925, the Royal Commission on Agriculture reviewed the production and distribution of seeds in India, it made several notable observations and valuable recommendations. Other committees/ persons who reviewed and made recommendations for the seed situation in India are as follows : Sir John Russel (1937), Imperial Council of Agricultural Research (1940), Dr. Barnes (1944), the

CHAPTER 28

Quality Seed : Classes, Production Practices and Maintenance

The primary objective of plant breeding is to develop superior varieties. The benefits from superior varieties can only be realised when they are grown commercially on a large scale. Seeds of improved varieties must be multiplied at a large scale in order to make them available to farmers for commercial cultivation. Here, *seed means seed or any other propagating material used for raising a crop,* and it does not mean the particular plant part botanically known as seed. For example, grain produced for consumption is not seed ; only grain produced for raising a crop would be called seed. On the other hand, potato tubers produced for planting a new crop are known as seed potatoes. During the multiplication of varieties for use as seed, it is essential that genetic purity of the variety must be maintained. If the genetic purity is not maintained, superiority of the variety is likely to be lost. In addition, for best results the farmer should use new pure seed every few years in case of self-pollinated crops, and every year (hybrid varieties) or every few years (composite and synrhetic varieties) in case of cross-pollinated crops. This would require the maintenance of seeds of superior varieties in genetically pure state, which would be multiplied every year to supply new seed to the farmers

The value of pure improved seed can hardly be overemphasised. The yielding ability and other characteristics of a variety are governed by its genotype, provided the environment is not limiting. Whether a farmer produces a good crop or a bad one with the help of all the inputs at his command largely depends on the variety he has chosen for cultivation. Pure seed of a superior variety would mean a good crop harvest, while contaminated seed may lead to crop failure. The seed may be contaminated by weeds, diseases or insects and may have poor germination. Use of such seeds would lead to poor crop stands, disease and pest problems and finally poor yield. Clearly, the seed of new varieties should reach the farmers in a pure and healthy state. To ensure this, elaborate seed programmes exist in most of the countries. India also has a well organised seed production programme in the form of National Seeds Corporation (NSC), State Seeds Corporation (SSC) and State Seed Certification Agency (SSCA). These organisations are responsible for seed certification and distribution, in essence for production and distribution of high quality seed. In a seed production programme, there are

strain is included in URT till it is released as a variety. Based on these data, the breeder submits a proposal for release of the variety before the Central Variety Release Committee. After release of a new variety, Ministry of Agriculture, Government of India, issues a notification for seed multiplication and distribution of the variety in the zone for which it has been released.

Each state has a State Variety Release Committee for release of varieties for that state only. Data from a new set of trials, in addition to those from coordinated trials, are required by the State Variety Release Committee.

QUESTIONS

1. Briefly describe the various steps involved in release of a new variety by the Central Variety Release Committee.
2. Write short notes on the following : (i) IET, (ii) URT, (iii) Agronomic trial, (iv) model agronomic trial, (v) CVT, (vi) National trial, (vii) Adoptive research trial, (viii) Minikit trial, (ix) disease tests, (x) quality tests, (xi) identification of a variety.
3. Compare the procedure of evaluation of new strains for release by Central and State Variety Release Committees. Which of the two appears to be more accurate and why ?

of Agriculture of the State (Chairman) ; (2) Director, State Seeds Corporation ; (3) Director, State Seed Certification Agency ; (4) Additional Directors of Agriculture of the State ; (5) Joint Directors of Agriculture of the State ; and (6) Directors of Research of all the Agriculture Universities of the State. In addition, (7) Deputy Directors of Agriculture of the State ; (8) Officers-in-Charge of all Regional Agricultural Testing and Demonstration Centres ; (9) Breeders, Agronomists, Pathologists and Entomologists of all Agriculture Universities and Agricultural Institutes in the state are also invited. It may be noted that the persons listed under items 7, 8 and 9 are, in fact, not the members of the committee, but they are invited to assist the committee in its deliberations.

Multiplication

When an entry is identified by the workshop of the concerned project, the breeder begins seed multiplication of that strain in the following crop season. The seed produced by the breeder after a strain is identified but before it is released as a variety is termed as the *stock seed*. The stock seed is known as *breeder seed* once the identified strain is released and notified. In the crop season following notification, foundation seed of the newly released variety is produced. In the second crop season following the release of a variety, certified seed of the variety is produced. Thus, the farmer is able to obtain certified seed of a newly released variety for commercial cultivation only in the third crop season following the release and notification of the variety. However, the seed of such a variety reaches many farmers even before its release (in the crop season following identification of the strain by the workshop) through Minikit Trials ; many other farmers would obtain the seed from these farmers (who conducted the Minikit Trials) and so on in the next two years. Thus an outstanding new variety is likely to reach many farmers even before its certified seed has been put on sale.

Multiplication and distribution of high quality seed of improved varieties is a crucial step in the agricultural production of any country. These aspects have been discussed in some detail in the following chapter (Chapter 28).

SUMMARY

A breeder evaluater new strains he has developed in station trials Outstanding strains are included in trials conducted by the All India Coordinated Crop Improvement Projects on a zone basis. In the first year, the new strains (called *entries*) are tested in Initial Evaluation Trial. Outstanding entries are promoted to Uniform Regional Trials (URT) in the second year. In the third year, outstanding entries from the first year URT are retained in URT and are also included in Agronomic Trials of the respective crop improvement projects. Based on the data of the second and the third years (that is, of URT) the entry may be proposed for identification for prereleases multiplication at the annual workshop of the respective coordinated projects. In the fourth year, the entries identified for release are evaluated in Adoptive Research Trials and Model Agronomic Trials. Disease tests are carried out from the first year in IET to the end of evaluation in URT. Quality tests are conducted from the time the

Release of A Variety by State Variety Release Committee

In addition to the Central Variety Release Committee, each state has its own variety release committee. The State Variety Release Committees usually require a separate set of trials conducted at the Regional Research Stations of the state, in addition to the data from coordinated multilocation trials. The procedure for testing of strains in the State of Uttar Pradesh is briefly summarised below.

First Year. The new entries are evaluated under one of the following three conditions : (*i*) timely sown, irrigated ; (*ii*) late sown, irrigated; and (*iii*) timely sown, rainfed.

Note. The plot size for these trials is 5×1.84 m and the number of replications is 4 to 6. Outstanding strains are promoted to the second year in trial.

Second and Third Years. The promising entries are evaluated under the same condition under which they were evaluated in the first year of trial. In addition, the strains are also included in an Agronomic Trial designed to determine the optimum date of sowing and the optimum level of fertilizer application.

Note. After the above evaluation, a strain may be proposed for release by the State Variety Release Committee.

The requirement of three-year evaluation at the Regional Agricultural Testing and Demonstration Centres of the concerned state for release of varieties by a State Variety Release Committee has some merit. The various locations within a state may not have been adequately represented in the evaluations under coordinated projects. In addition, parts of a single state may be included in different zones of evaluation in the coordinated projects. Thus evaluation at a number of locations within a state gives a more clear picture of the adaptation of a variety in different zones within a state. However, it does delay the release of a variety by a couple of years and this may, sometimes, hinder the spread of an otherwise outstanding variety.

A variety released by a State Variety Release Committee must be notified by the Ministry of Agriculture, Government of India before its certified seed can be produced. Further, only those varieties are notified by the ministry which have been identified for prerelease multiplication by the workshop of the concerned coordinated project. A state Variety Release Committee, however, may release a strain as a variety which has not been identified by the workshop of the concerned coordinated project (or even such strains which were not evaluated under the coordinated projects). But such varieties cannot be notified, and certified seed of such varieties cannot be produced.

Constitution of The State Variety Release Committee. The State Variety Release Committee consists of the following : (1) Director

notification of the variety. A variety must be notified by the Ministry of Agriculture, Government of India, before its seed can be certified.

The various trials conducted for evaluation of wheat strains before release are summarised below yearwise.

First Year

1. Initial Evaluation Trial (IET) (timely sown, irrigated ; late sown, irrigated ; and timely sown, rainfed).
2. Disease tests.

Note. Outstanding entries promoted to URT, the rest are rejected.

Second Year

1. Uniform Regional Trials (URT) (timely sown, irrigated ; late sown, irrigated ; and timely sown, rainfed).
2. Disease Tests.
3. Quality Tests.

Note. Outstanding entries retained in URT and included in Agronomic Trials ; poor entries rejected.

Third Year

1. Uniform Regional Trials (as in the second year).
2. Agronomic Trials (dates of sowing ; and number of irrigations : one, two and adequate).
3. National Trials (top-ranking entries in URT from each zone pooled ; serves as IET for other zones).
4. Disease Tests.
5. Quality Tests.

Note. Outstanding entries proposed for identification ; proposals for identification submitted by the concerned breeders at the annual workshop of the concerned coordinated project. Remaining entries are rejected.

Fourth Year

1. Adoptive Research Trials
2. Model Agronomic Trials
3. Disease Tests
4. Quality Tests

Note. The breeder may propose for release of the variety. The proposal is considered by the Central Variety Release Committee If the variety is released, the Ministry of Agriculture and Irrigation, Government of India issues a notification for seed multiplication and distribution of the variety.

Table 27.3. Proforma for the submission of proposal for the notification of a variety under Section 5 of the Seeds Act, 1966 (180 copies of this proposal are submitted to the Ministry of Agriculture and Irrigation, Government of India)

1. State :
2. Crop :
3. Name of the variety under which released :
4. Year of release :
5. (a) Parentage with detail of its pedigree :
   (b) Source of material in case of introduction :
   (c) Breeding method :
   (d) Breeding objective :
6. State the varieties which most closely resemble the proposed variety in general characteristics :
7. (a) Breeder/Institute responsible for maintaining breeder's stock :
   (b) Quantity of breeder's seed of the variety available (in kg) :
8. Description of variety/hybrid :
9. Description of the parents of the hybrid. Is there any problem of synchronization ? If yes, methods to overcome it :
10. Describe at least two identifiable and distinguishable morphological characteristics of the variety. In case of hybrid, please describe also at least two identifiable and distinguishable morphological characteristics of both the parents :
11. Maturity group (early, medium and late, wherever such a classification exists) :
12. Disease and pest resistance (give details of any resistance to pests or diseases including races) :
13. Recommended ecology ;
14. Yield (in kg/hectare)
    (a) Commercial product :
    (b) Seed :
15. Current approximate percentage of the area of the crop (kind) under this variety in the State ;
16. Recommendation of the All India Workshop about ihe variety :

Signature of the Chairman/Convenor,
State Seed Sub-Committee
Name
Designation

identification are very rigid. A cotton variety (other than hybrid) must outyield the check variety by 10-20%, while a hybrid must have 30% superiority in yield over the check for being identified ; further, they must be at least comparable to the check in disease and insect resistance. These criteria are fixed by the workshop of the concerned coordinated projects and reflect the experiences of the workers with the concerned crops. As a result, they are not fixed entities, and are likely to vary as per the needs of the nation as well as the experiences of the concerned workers.

In addition, one of the essential requirements for identification of a strain is that the breeder must be able to spare 10 Q seed of the proposed strain. This seed is supplied to State Farms Corporation of India (SFCI) for prerelease multiplication as well as for distribution for adoptive and minikit trials.

Release of A Variety by The Central Variety Release Committee

After identification, a variety is tested for at least one year in Adoptive Research Trials. During this period, disease tests and quality tests are also conducted. Based on the data from adoptive trials and disease and quality tests, the breeder who has developed the concerned strain submits a proposal for its release as a new variety for consideration by the Central Variety Release Committee. The proposal is prepared according to a prescribed proforma (Table 2.73) giving full details about the pedigree and the breeding method used for developing the strain and the performance of the strain in the various trials, disease and quality tests, adoptive trials etc. The breeder usually gives the variety a name or he may give it a number, which is included in the proposal.

The Central Variety Release Committee consists of the following : (1) Deputy-Director General (Crop Sciences), ICAR, New Delhi (Chairman) ; (2) Production Commissioner, Government of India ; (3) Project Director/Project Coordinator of the respective crop improvement project ; (4) Principal Investigators for all the disciplines of the concerned coordinated project ; (5) Directors of Agriculture of all the states (the presence of Directors of Agriculture of the states within the zone for which a variety is to be released is essential) ; (6) a representative of the National Seeds Corporation; (7) Director, High Yielding Varieties, Ministry of Agriculture and Irrigation, Government of India ; (8) Deputy Secretary (seeds), Ministry of Agriculture and Irrigation, Government of India ; and (9) Project Coordinator, All India Coordinated Agronomic Research Project.

After a variety has been released for a zone by the Central Variety Release Committee, the Director, High Yielding Varieties, Ministry of Agriculture and Irrigation, Government of India, notifies the concerned authorities of the states within that zone for seed multiplication and distribution of the variety ; this is known as

for various uses. For this reason, quality tests are generally done in certain specialised laboratories well-equipped for the purpose. The Quality tests are generally carried out on all the entries included in URT/CVT.

Quality tests of wheat are done at the quality laboratories of IARI, New Delhi : PAU, Ludhiana ; and G.B. Pant University of Agriculture and Technology, Pantnagar. Similarly, quality of promising new cotton strains is evaluated in Cotton Technological Research Laboratory, Matunga, while the quality of promising new strains of jute is tested in Jute Technological Research Laboratory, Calcutta. The following tests are conducted to assess the quality of wheat strains : (1) protein content, (2) Polshenke test, (3) chapati making quality test, (4) bread making quality test, (5) mixograms, (6) alveographs, and (7) sedimentation value.

Identification of Entries for Release

Outstanding strains are identified for release as new varieties at the annual workshop of the coordinated projects on the respective crops. The proposals for identification in some crops are considered in an open forum by the participants in the workshop, while in some other crops, *e.g.*, wheat, they are examined by an Identification sub-committee, which ordinarily has the following composition : (1) Deputy-Director General (Crop Sciences), ICAR, New Delhi (Chairman) ; (2) the Project Director/Project Coordinator of the concerned Crop Improvement Project ; (3) Principal Investigators for the different disciplines, *e.g.*, Agronomy, Pathology, Quality Entomology, Physiology etc., of the concerned coordinated project ; and upto 5-6 persons actively involved in the project.

Proposals for the identification of entries are prepared by the respective breeders who have developed the concerned strains. They are prepared according to a prescribed proforma and include the performance of the entries in URT for at least two years, data from pathological, entomological, quality tests etc., and those from Agronomic Trials.

An entry considered suitable for release as a variety by the concerned workshop is said to have been *identified for prerelease multiplication* or simply as *identified*. The criteria for identification of enteries vary considerably from one crop to the other. In wheat, an entry may be identified if it falls into one of the following two categories : (1) an entry significantly superior to the check in yield and comparable to the check in disease resistance, and (2) an entry comparable to the check in yield but consistently and markedly superior to it in disease resistance. However, an entry immune to diseases but significantly inferior to the check in yield is not identified since yield is the most important breeding objective in any crop. In comparison, entries of oilseed crops must show 10% superiority in yield over the check before they can be identified by the workshop. Cotton provides an extreme example where requirements for

400 places within a zone. In wheat, five kilograms of seed of each variety is planted without any replication ; the agronomic practices are those for which the variety is identified for release. The objective of Minikit Trials is to popularise the new variety among farmers of the zone. It also serves another purpose ; the seed of a good new variety reaches the farmers one year earlier than when its certified seed would be available in the market.

Disease And Insect Tests. Entries are evaluated for disease and insect resistance throughout the period of testing, that is, during IET as well as URT. Disease and insect resistance is tested both under natural epidemic as well as artificial epiphytotic (epidemic) conditions. The disease reaction tests for different diseases of various crops are carried out at different places where epidemics of the concerned disease occur regularly. The places where a disease occurs at a high intensity, that is, the disease occurs in an epidemic form, every year are known as *hot spots* for that disease. The hot spots for some of the common wheat diseases are as follows : (1) black rust (stem rust), Peninsular Zone ; (2) leaf rust (orange rust), all zones ; (3) stripe rust (yellow rust), Northern Plains Zone ; and (4) Karnal bunt, Ludhiana (Punjab). Dhaula Kuan (H.P.), Pantnagar (U.P.) and Gurdaspur (Punjab). The disease reaction of various entries is evaluated by pathologists at the respective centres. In addition, the disease reaction is monitored throughout the zone wherever the trials are conducted. For testing disease resistance under artificial ephiphytotic conditions, specialised laboratories have been established. Resistance to wheat leaf and stripe rusts is tested (under artificially created epidemics) at Simla, while that to stem rust is tested at Delhi, Indore and Mahabaleshwar centres.

The value of disease tests cannot be overemphasized. In an extreme case, susceptibility of released variety to a disease may spell disaster to the farmer, and sometimes, may even cause health hazards to the consumers as well. The early hybrid bajra varieties (*e.g.*, HB1, HB 2) are a case in point. These hybrids were extremely susceptible to downy mildew and ergot. In a year of severe epidemic, the whole produce had to be destroyed due to the hazard to consumer health posed by ergot infected grains. In any case, susceptibility to a disease would lead to economic losses to the farmers, and often would lead to the rejection of a variety. In view of these, disease resistance of a strain is evaluated during each year in which a strain included in various yield trials.

Quality Tests. Quality tests are conducted to determine the suitability of an entry for various uses of its produce. For example, in case of wheat, the *chapati* and bread making qualities of grains are important for their acceptance by consumers. The quality of a crop is not a simple character, and is often not easy to determine. Therefore, a number of different tests have to be conducted for which sophisticated equipments may be needed for speedy and reliable results. In each crop, a different set of quality tests have been developed in order to determine the suitability of new strains of the crop

respective All India Coordinated Crop Improvement Projects. The purpose of Agronomic Trials is to determine the suitable dates of sowing and the optimum number of irrigations. The plot size in wheat Agronomic Trials is 10×1.61 m and the number of replications is 3. In wheat, two types of experiments are conducted : varieties $\times$ dates of sowing, and varieties $\times$ number of irrigations. Three dates of sowing are used with each variety ; early (last week of October), normal (15-25 November) and late (second week of December). The three levels of irrigation are : one irrigation, two irrigations and adequate number of irrigations. The data from these trials are generally not essential for the consideration of an entry for identification, although they are included in the proposals for varietal identification.

4. Model Agronomic Experiments. These experiments are conducted under the All India Coordinated Agronomic Research Project (and not under the coordinated project for improvement of the concerned crop). In Model Agronomic Trials, only those entries are included that have been identified by the workshop of the respective coordinated projects. These trials are conducted at all the centres of the Coordinated Agronomic Research Project in the zone.

National Trials. The national trials are conducted throughout the country in all the zones. The entries in National Trials consist of one entry from each zone that ranked first in that zone in URT of the previous year. The National Trials serve as IET for an entry in zones other than that in which it was developed and tested in URT. The purpose of National Trials is to evaluate outstanding entries of one zone in the other agroclimatic zones to see if they perform well in other zones as well. It has been suggested that National Trials should include IET entries of all the zones so as to evaluate all entries on a national level. This is undoubtedly desirable in the identification of entries adapted to various zones, but it would increase the number of entries in the national trials to an unmanageable level in a crop like wheat.

Adoptive Research Trials. Adoptive Research Trials are conducted on research stations or farms of state governments. The entries identified by the workshop of the concerned coordinated project are included in these trials. The plot size is 0.5 acre (0.2 hectare) and there is no replication. The agronomic practices used in Adoptive Research Trials are the same for which the concerned entry has been identified. The data from these trials are considered for release of the identified entry as a new variety. Thus, each identified entry has to undergo adoptive trials before it is released as a variety.

Minikit Trials. The Minikit Trials are conducted in the farmer's fields. These trials are conducted alongwith the Adoptive Trials in the following crop season when the entry is identified for release. The Minikit Trials are conducted under the supervision of Director, High Yielding Varieties, Ministry of Agriculture and Irrigation, Government of India. The Minikit Trials are conducted at 300 to

for identification in the annual workshop of the coordinated wheat improvement project. If the entry is identified for release, it is evaluated in Adoptive Research Trials, Model Agronomic Trials and Minikit Trials. Thus, data from evaluation for two years in URT and one year in adoptive trials is essential for the release of a wheat entry as a variety. The data from Agronomic Trials, Model Agronomic Trials and Minikit Trials are not essential for the release of a variety, although those from agronomic trials are included in the release proposal. Thus *wheat entries are evaluated for one year in IET and two years in URT before their identification by the workshop of the project for prerelease multiplication and for adoptive trials ; identified entries are tested for one year in adoptive trials before their consideration for release as a variety by the Central Variety Release Committee.*

The duration of different trials in various crops varies to some extent. In most pulses, except gram and mungbean, there is no IET and URT ; the entries are directly included in CVT (Coordinated Varietal Trial) and evaluated for three years. In Jowar, the evaluation consists of one year of Preliminary Sorghum Trial, three years of Advanced Sorghum Trials and two years of Adoptive Trials. In Oilseeds, the entries are evaluated for one year in IET, one year in CVT, one year in NET (National Evaluation Trial) and one year in AT (Adoptive Trials). In NET, the plot size is three-times the size of that in CVT ; data from one year IET, one year CVT and one year NET are required for the identification of an entry for prerelease multiplication.

The criteria for the promotion of an entry from IET to URT (or CVT), for the retention for evaluation in the second/third year URT/CVT, and for the identification of entries by the workshop vary from one crop to the other. In case of wheat, only those entries are promoted from IET to URT or retained in URT which belong to the top nonsignificant group in terms of yield ; the top nonsignificant group consists of those entries whose yields are not significantly inferior to the yield of the highest yielding entry in the given zone. In addition, such entries must have an acceptable level of resistance to diseases (mainly rusts) ; the average coefficient of infection (ACI) must be less than 15 under irrigated and less than 20 under rainfed conditions. Similarly, acceptable levels of resistance to important diseases and pests of other crops have been defined. But in some crops, resistance to diseases and/or pests may not be an important criteria for promotion/retention of entries in trials. For example, in the case of rapeseed and mustard all the trials are conducted under plant protection umbrella chiefly because entries resistant to the main diseases (white rust, *Alternaria* leaf blight) and insects (aphids) are not available. Similarly, in vegetable peas a separate evaluation of disease (powdery mildew) susceptible entries is carried out (Table 27 2).

3. Agronomic Trials. An entry showing superior performance in URT during the first year is included in Agronomic Trials of the

It should be noted that zones have not been created on the basis of political boundaries, but on the basis of agroclimatic conditions prevailing in the area. Thus, it may be expected that the agroclimatic conditions at any two locations within a zone would be more comparable than those at two locations in any two zones.

The various trials conducted under the coordinated projects, particularly in the case of wheat, may be grouped into the following four categories : (1) initial evaluation trial (IET), (2) uniform regional trials (URT), (3) agronomic trials, and (4) model agronomic experiments.

1. *Initial Evaluation Trial* (*IET*). A new strain is first included in an initial evaluation trial within the zone in which it was developed. A strain included in IET or URT is commonly termed as an *entry* ; this term will be used in the above sense in subsequent discussions. The IET is conducted at 10-12 locations within a zone. The plot size in wheat IET is 6×1.38 m, and the number of replications is generally 6. In wheat, IET is conducted under the following three conditions : (1) timely sown, irrigated ; (2) late sown, irrigated ; and (3) timely sown, rainfed ; URT is also conducted under the same three conditions. The conditions under which new strains of various crops are evaluated are summarised in Table 27.2. In most crops, the conditions of evaluation vary from two to four. But in arhar, there are five different evaluation conditions, while in peas the strains are tested under nine different conditions. A strain is normally included in only one of the above three types of IET of wheat. *The entries are tested in IET for one year only ; they are promoted to Uniform Regional Trials (URT) if their performance is outstanding, otherwise they are rejected.* In case of some crops, *e.g.*, pulses and oilseeds, an entry may be tested in IET for more than one year. An outstanding entry promoted to URT is tested under the same condition in which it was evaluated in IET. The objective of IET is to eliminate relatively inferior entries so that their number is reduced to a manageable size for URT evaluations.

2. *Uniform Regional Trials* (*URT*). The entries that are outstanding both in yield and disease resistance in the IET of the previous year are promoted to the Uniform Regional Trials. The URT is conducted under the same condition under which the concerned entry was evaluated in IET. URT is conducted at 25-30 different locations within the zone. The plot size in wheat URT is 6×2.76 m and the number of replications is usually 6.

URT may be continued for one or more years. Every year, based on the performance in URT, entries poor in yield and/or disease resistance are rejected. New entries are also included on the basis of their performance in IET of the previous year. After evaluation for one year in URT, the outstanding entries are included in Agronomic Trials, and their evaluation in URT is continued for one more year. In the case of wheat, after evaluation for two years under URT entries superior in yield and disease resistance may be proposed

Crop	Zones*	Evaluation procedure**	Conditions of testing
Lentil	5 (NWHZ, NWPZ, NEPZ, EZ, CZ)	CVT, 3 Yrs	1. Small-seeded, timely sown 2. Bold-seeded, timely sown (both rainfed ; irrigation if required)
		Oilseeds	
Mustard	5 (HZ, NWZ, CZ, WZ, EZ)	LET, 1 Yr CVT, 1Yr NET, 1 Yr AT, 1Yr	1. Timely sown, rainfed 2. Timely sown, irrigated
Toria	—do—	—do—	1. Early maturity, irrigated 2. Late maturity, irrigated 3. Rainfed
Yellow sarson	—do—	—do—	1 Timely sown, irrigated 2. Timely sown, rainfed
Brown sarson	—do—	—do—	Rainfed
Groundnut	6 (NZ, WZ, CZ, SEZ, SCZ, SZ)	—do—	A. 1 Rabi, irrigated B. Kharif, rainfed 2. Bunchy 3. Semispreading 4. Spreading
Linseed	4 (NWZ, Indo-gangetic alluvium, CZ, PZ)	—do—	1. Timely sown, irrigated 2. Timely sown, rainfed
Safflower	2 (Traditional : Maharashtra, AP, Tamil Nadu ; Non-traditional : the rest of the country)	—do—	1 Rainfed 2. Irrigated; only at Phaltan (Maharashtra ; 2 irrigations)
		Cash Crop	
Soybean	4 (NHZ, NPZ, CZ, SZ)	IVT, 1 Yr AVT, 1 Yr ZET, 2 Yrs	1. Irrigated (all zones) 2. Rainfed (AVT and ZET in CZ and SZ)
Cotton	3 (NZ, CZ, SZ)		**A. Desi Cotton** 1. Varieties 2. Hybrids (*G. harbaceum* × *G. arboreum*) **B. American Cotton** 3. Varieties : Early maturity 4. Varieties : Late maturity 5. Hybrids (CZ and SZ only)

* **NHZ**, Northern Hills Zone; **NPZ**, Northern Plains Zone; **NWPZ**, North-Western Plains Zone; **NEPZ**, North-Eastern Plains Zone; **EPZ**, Eastern Plains Zone; **FEZ**, Far-Eastern Zone; **CZ**, Central Zone; **PZ** Peninsular Zone; **SHZ**, Southern Hilis Zone ; **NWHZ**, North-Western Hills Zone; **NEHZ**, North-Eastern Hills Zone; **WZ**, Western Zone; **SEZ** South-Eastern Zone; **NEHZ**, North-Eastern Hills Zone; **HZ**, Hills Zone, **NWZ**, North-Western Zone; **NZ**, Northern Zones; **SCZ**, South-Central Zone; **SZ**, Southern Zone.

** **IET**, Initial Evaluation Trial; **URT**, Uniform Regional Trial; **AT**, Adoptive Trial; **PST**, Preliminary Sorghum Trial; **AST**, Advanced Sorghum Trial; **CVT**, Coordinated Varietal Trial; **NET**, National Evaluation Trial (plot size three-rimes the size of that in CVT; in oilseeds entries identified for prerelease multiplication after NET); **PVT**, Preliminary Varietal Trial; **UVT**, Uniform Varietal Trial. **ITV**, Initial Varietal Trial; **AVT**, Advanced varietal trial; **ZET**, Zonal Elite Trial.

Crop	*Zones**	*Evaluation procedure***	*Conditions of testing*
		Millets	
Maize	6 (Zones 1, 2, 3, 4, 5, and 6)	Stage, 0 (Zonal) Stages 1-3 (All India basis)	A. Late maturity (>90 days) B. Medium maturity (85-90 days) C. Early maturity (80-85 days) D. Extra Early (80 days)
Jowar	1 (the whole of India)	PST, 1 Yr AST, 3 Yrs AT, 2 Yrs	1. Hybrid sorghum trials 2. Varietal sorghum trials
Bajra	1 (The whole of India)	UVT, 3 Yrs AT, 1 Yr	1. Bajra varieties trials 2 Bajra hybrid trials
		Pulses	
Arhar	7 (NWHZ, NEHZ, NWPZ, WZ, NEPZ, EZ, CZ) (EXACT and EACT, 7 Zones; ACT-1 and ACT-2, 6 Zones; ACT-3, 2 Zones)	CVT, 3 Yrs	1. Extra early (EXACT, maturity 100 days), rainfed. 2. Early (EACT, 100 130 days), rainfed. 3. Normal (ACT-1, 130-160 days) 4. Medium (ACT-2, 160 200 days) 5. Late (ACT-3, >200 days) (ACT-1, -2, -3; if needed, one irrigation at flowering)
Gram	9 (NWHZ, NWPZ, WZ, NEPZ, EZ, SEZ, CZ, SZ, NEHZ) (Late sown, 7 zones; bold-seeded, 7 zones; Kabuli, 4 zones)	IET, 1 Yr CVT, 2 Yrs AT, 1 Yr (In categories other than timely sown, no IET; CVT, 3 Yrs)	1. Timely sown, irrigated 2. Late sown, irrigated 3. Bold-seeded (>20g 100-seed weight), irrigated (all desi-types) 4. Kabuli, timely sown, irrigated
Peas	7 (NWHZ NWPZ, EZ, NEPZ, CZ, NEHZ, WZ) (Dwarf trial, 5 zones; vegetable peas, single zone)	CVT, 3 Yrs	A. **Field peas (white-seeded, timely sown, irrigated)** 1. Normal height 2. Dwarf B. **Vegetable peas (green-seeded)** 3. Susceptible, early maturity 4. Susceptible, medium 5. Susceptible, late 6. Resistant, early 7. Resistant, medium 8. Resistant, late
Mungbean	8 (NWHZ, NEHZ, WZ, CZ, NWPZ, NEPZ, SEZ, SZ) (Spring, 7 zones; summer, 1 zone).	IET, 1 Yr CVT, 2 Yrs AT, 1 Yr (Spring: CVT, 2 Yrs; Summer: CVT, 3 Yrs).	1. Kharif, timely sown, irrigated/rainfed 2. Spring, timely sown, irrigated 3. Summer, timely sown, irrigated
Rajma	2 (NEPZ, CZ)	CVT, 3 Yrs	Timely sown, irrigated

Similarly, only two crops (wheat and maize) were covered in 1966-67, while quality seeds of more than 70 crops are now being produced. The values quoted in Table 28.1 do not include the quantity of seed produced and marketed by private seed companies

THE INDIAN SEED ACT (1966)

The Indian Seeds Act was enacted in 1966, and has been in force since Oct. 2, 1969 in all the States and Union Territories of the Indian Union. This act aims at regulating the quality of seed sold for agricultural purposes through compulsory labelling and voluntary certification. Under *compulsory labelling*, anyone selling the seed of a notified kind or variety, in the region for which it has been notified, should ensure that (1) the seed conforms to the prescribed limits of germination and purity, (2) the seed container is labelled in the prescribed manner, and (3) the label truly represents the quality of the seed in the container. Under *voluntary certification*, any one interested in producing certified seed may do so by applying to the seed certification agency for the grant of a

Table 28.1. The growth of seed industry in India as indicated by the quantities of quality/certified seed produced in some important crops. (Based on, R L. Agrawal, 1975 ; and Anonymous, 1987. Agricultural Statistics at a glance, Ministry of Agriculture, Govt. of India).

Crop	*Quantity of quality/certified seed (in lakh quintals)*				
	1966-67	*1967-68*	*1973-74*	*1980-81*	*1984-85*
Wheat	0.24	0.83	1.49	10.17	12 09
Rice	—	0.05	0.57	6.41	8.49
Maize	0.12	0.13	0.08	0.83	1.51
Jowar	—	—	—	1.51	2.21
Bajra	—	—	—	1.04	1.59
Other cereals	—	—	—	—	0.22
Gram	—	—	—	—	0.66
Urd	—	—	—	—	0.32
Pea's	—	—	—	—	0.23
Other pulses	—	—	—	1.06*	0.88
Groundnut	—	—	—	—	4.67
Rape/mustard	—	—	—	—	0 49
Soybean	—	—	0.02	—	0 90
Other oilseeds	—	—	—	2.28**	0.47
Cotton	—	—	—	—	1.19
Jute	—	—	—	—	0.22
Potato	—	—	—	1.69	12.25***
Others	—	0.002	0.06	—	0.07
Total	0.36	0.998	2.22	25 01	48 46

*Total for all the pulses ; crop-wise quantities not available.
**Total for all the oilseeds.
***Estimated.

certificate. The agency grants the certificate and certification tags after satisfying itself that the seed has been produced according to the prescribed standards and procedures. There are two bodies, *viz.*, The Central Seed Committee and the Central Seed Certification Board, which advise the central and the state governments in the matters related to the general administration of the seeds act and of seed certification, respectively.

CLASSES OF QUALITY SEED

The various classes of seed that are used in a seed production programme are : (1) breeder seed, (2) foundation seed, (3) registered seed, and (4) certified seed. These classes of seeds were first clearly defined by the International Crop Improvement Association in 1946 in relation with fodder and forage crops ; in 1968 it recommended the adoption of the same system in the case of grain crops as well. These different classes of seeds have different requirements and serve different functions, a brief description of which is given below.

Breeder Seed. Breeder seed is the seed or the vegetative propagating material produced by the breeder who developed the particular variety. It is produced by the institution where the variety was developed in case the breeder who developed the variety is not available. In India, Breeder seed is also produced by other Agriculture Universities under the direct supervision of the breeder of the concerned crop working in that University; this arrangement is made in view of the large quantities of the breeder seed required every year. Breeder seed is used to produce the foundation seed.

Breeder seed is genetically pure. In case of self-pollinated species, mass selection is regularly practised to retain the genetic purity of the variety. Off-type plants are promptly eliminated and care must be taken to prevent outcrossing or natural hybridization and mechanical mixtures.

Foundation Seed. Foundation seed is obtained from breeder seed by direct increase. Foundation seed is genetically pure and is the source of registered and/or certified seed. Production of foundation seed is the responsibility of NSC. Foundation seed is produced on Government farms, at experiment stations, by Agriculture Universities or by competent seed growers under strict supervision of experts from NSC. This class of seed should be produced in the area of adaptation of the concerned variety.

Registered Seed. Registered seed is produced from foundation seed or from registered seed. Registered seed is genetically pure and is used to produce certified seed or registered seed. Registered seed is usually produced by progressive farmers according to technical advice and supervision provided by NSC. Often registered seed is omitted and certified seed is produced directly from foundation seed. This is the general practice in India.

Certified Seed. Certified seed is produced from foundation, registered or certified seed. This is so known because it is certified by a seed

certification agency, in this case State Seed Certification Agency, to be suitable for raising a good crop. The certified seed is annually produced by progressive farmers according to standard seed production practices. To be certified, the seed must meet certain rigid requirements regarding purity and quality. These standards vary from one crop to another and shall be discussed later in some detail. Certified seed is available for general distribution to farmers for commercial crop production. Its production is generally by State Seeds Corporations, but NSC also undertakes the supervision of certified seed production, if required.

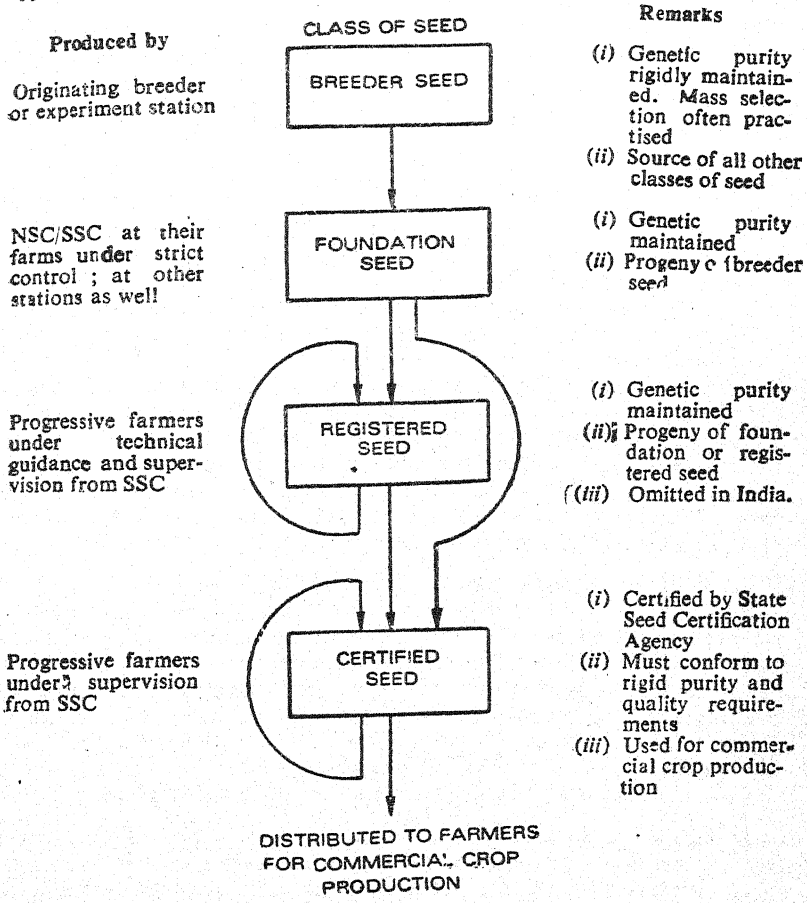


Fig. 28.1. Steps in seed multiplication.

The various classes of improved seed are recognised to facilitate the maintenance of genetic purity of the variety and to ensure a continuous supply of good quality seed at a reasonable cost. It also

helps in multiplication of the seed rapidly while maintaining the purity of the seed. The production of breeder and foundation seeds is very costly since a very high standard of purity must be maintained. The requirements for certified seed are relatively less rigid than those for foundation seed, hence it is considerably cheaper. The various steps in seed multiplication are outlined in Fig. 28.1.

Sometimes, the seed produced under unfavourable weather conditions may meet the requirements of purity etc. but may be smaller in size and poorer in germination. Such seed may be classified as *substandard seed*, and issued tags to that effect. Substandard seed has to fulfil the requirements for physical and genetic purity, and freedom from weed seeds and diseases as prescribed for certified seed. Such a seed may be used when standard certified seed is not available for growing commercial crops.

REQUIREMENTS FOR CERTIFIED SEED

Seed has to meet certain rigid requirements before it can be certified for distribution. *The first and foremost requirement is that the seed must be of an improved variety released by either the Central or a State Variety Release Committee for general cultivation and notified by the Ministry of Agriculture, Government of India ; this is essential for the seed to be certified.* The other requirements are related to genetic purity, freedom from weeds, diseases and pests, germination etc. The various requirements for certified seeds are summarised in Table 28.2 for some of the field and vegetable crops. It may be noted that there is considerable variation in the requirements for certification in various crops. In certain cases, *e.g.* maize (*Z. mays*), the requirements are more rigid than in the others. These requirements are briefly described below.

Genetic Purity. *Genetic purity means the absence of seeds of other varieties of the same crop species as well as of other crop species.* Genetic purity ensures that the seed is of the variety under certification, and that there is no mixture from other varieties or other crops. The standard of genetic purity is very high ; the amount of contamination permitted ranges from 0-0.1 per cent. *In most of the cases, contamination by seeds of other crop species is permitted to a small degree (up to 0.1 per cent), but contamination by seeds of other varieties of the same crop is generally not permitted (Table 28.2).*

Physical Purity. *Physical purity implies freedom of seed from inert matter and defective seed.* Inert matter consists of nonliving materials such as sand, pebbles, soil particles, straw etc. Defective seeds are those seeds that are broken, diseased, insect infested, shrivelled and unfit for germination. A broken seed larger than half of the normal seed is not considered defective provided the embryo is not damaged. The total amount of permissible contamination by inert matter and defective seed ranges from 1.0 per cent in the case of maize (*Z. mays*) to 5 per cent in the case of carrot (Table 28.2).

Table 28.2. Germination and purity standards for certified seeds of different field and vegetable crops.

Crop	*Per cent of total seed (on weight basis)*							
	Pure seed (min.)	*Other crop seed (max.)*	*Other varieties (max.)*	*Total weed seed (max.)*	*Objectionable weed seed (max.)*	*Total inert matter (max.)*	*Moisture (max.)*	*Germination (min.)*
Field Crops								
Hybrid maize (other than single cross)	98	0.2	—	None	—	2	12	90
Maize composites and open-pollinated varieties	98	1.0	—	None	—	2	12	90
Hybrid jowar and varieties	98	0.1	—	0.1	None	2	12	80
Hybrid bajra* and open-pollinated varieties	98	0.1	—	0.1	—	2	12	75
Rice*	98	0.1	—	0.1	5 seeds/40 kg	2	13	80
Wheat*	98	0.1	—	0.1	5 seeds/kg	2	12	85
Barley	98	0.1	—	0.1	5 seeds/kg	2	12	85
Cotton varieties and hybrids	98	0.1	—	0.1	—	2	10	60
Gram	98	0.05	—	None	—	2	9	85
Arhar	98	0.2	—	0.2	—	2	10	75
Urid	98	0.2	—	0.5	—	2	9	65
Mung	98	0.2	—	0.1	—	2	9	75
Rapeseed and mustard	97	0.5	—	0.5	0.1	3	8	85
Sesamum (*til*)	97	0.2	—	0.2	—	3	9	80
Groundnut	96	None	—	None	—	4	9	70
Sunflower	98	None	—	None	—	2	9	60
Linseed	98	0.2	—	0.2	—	2	7	80
Soybean	97	0.05	0.5	0.10	—	3	12	70
Vegetables								
Peas	98	0.05	0.5	None	—	2	9	75
Cowpeas	98	0.05	0.2	0.1	—	2	9	75
Tomato	98	0.1	—	None	—	2	8	70
Cauliflower	98	0.1	—	0.2	—	2	7	65
Bhindi	99	0.05	—	None	None	1	10	65
Watermelon and other cucurbits	99	0.1	0.2	None	None	1	7	60
Onion	98	0.1	—	0.2	—	2	8	70
Carrot	95	0.1	—	0.2	—	5	8	60
Chillies	98	0.1	—	0.1	—	2	8	60
Radish	98	0.1	—	0.2	—	2	6	70
Brinjal	98	None	—	None	—	2	8	70

*Diseased seed standard (max.) : 1. Bajra, ergot sclerotia 0.04% (by number)
2. Wheat, nematode galls of tundu ear cockle or Karnal bunt 0.5% (by number)
3. Rice, paddy bunt 0.5% (by number)

Germination. A high percentage of germination is necessary to obtain a good crop stand with the minimum amount of seed. The percentage of germination required for certification is high in a crop like maize (90%), moderate in several others, *e.g.*, jowar (80%), wheat (85%), rice (80%) etc., and relatively low in many others, *e.g.*, cauliflower (65%), bhindi (65%), carrot (60%) and chilli (60%). Thus the minimum standard of germination for seed certification varies considerably from one crop to the other (Table 28.2).

Freedom from Weed Seeds. Freedom from weed seeds is necessary to prevent weeds from spreading through seed and to reduce losses caused by weeds. The maximum amount of weed seeds permitted is very low ; it varies from zero per cent in crops like maize (*Z. mays*) and tomato (*L. esculentum*) to 0.2 per cent in cauliflower (*B. oleracea*), onion (*A. cepa*), carrot (*D. carota*) etc. For some crop species, some weeds are classified as objectionable or noxious weeds. In such crops, the certified seed is required to be free from seeds of the noxious weed species.

Freedom from Diseases. The certified seed must be free from seed-born diseases. If the seed is contaminated with pathogens, it is likely to lead to an epidemic and a total loss of the crop. The presence of seed-born pathogens is prevented in two ways : *first*, by effective control of diseases in the standing crops, and *second*, by treating seeds with disinfectants and protectants. The maximum permissible frequency of diseased seeds is given below Table 28.2.

Optimum Moisture Content. The seeds must be dried to an optimum moisture level (Table 28.1) for efficient processing and safe storage. The level of optimum moisture varies from one crop species to another. It varies from 13 per cent in case of rice to 7 per cent in the case of cauliflower and watermelon (*C. vulgaris*). A moisture content higher than the recommended level (1) leads to a loss in seed viability, (2) promotes growth of microorganisms, particularly moulds, (3) favours attacks by storage insect pests, (4) increases the chances of damage to seeds by fumigants, and (5) interferes with processing because damp seeds tend to stick together.

REQUIREMENTS FOR FOUNDATION SEED

The foundation seed, like certified seed, has to fulfil a set of rigid quality requirements ; these standards are summarised in Table 28.3. The standards for pure seed (minimum), inert matter (maximum), moisture content (minimum) and germination (minimum) are comparable for foundation seeds with those for certified seeds of various field and vegetable crops. However, the maximum permissible limits for the quantities of other crop seed, seeds of other varities, total weed seed and seeds of objectionable weeds are much lower in the case of foundation seeds than those for certified seeds. For example, in the case of jowar the maximum permissible limits for other crops seed and total weed seed are 5 seeds/kg in the case of foundation seed, while they are 0.1% in the case of certified seed (Tables 28.2 and 28.3). Similarly, in the case of

Table 28 3. Germination and purity standards for foundation seeds of some field and vegetable crops.

Crop	*Pure seed (min.)*	*Other crops seed (max.)*	*Seeds af other varieties (max.)*	*Total weed seed (max.)*	*Objectionable weed seed (max.)*	*Total inert matter (max.)*	*Moisture (max.)*	*Germination (min.)*
Field Crops								
Maize	98.0	0.2, 1.0**	—	None	—	2	12	80, 90***
Jowar	98.0	5/kg	—	5/kg	—	2	12	80
Bajra	98.0*	10/kg	—	10/kg	—	2	12	75
Rice	98.0*	10/kg	—	10/kg	2/kg	2	13	80
Wheat	98.0*	10/kg	—	10/kg	2/kg	2	12	85
Barley	98.0	10/kg	—	10/kg	2/kg	2	12	85
Cotton	98.0	5/kg	—	5/kg	—	2	10	65, 60
Gram	98.0	None	—	None	—	2	9	85
Arhar	98.0	0.1	—	0.1	—	2	10	75
Urd and Mung	98.0	0.1	—	0.05	—	2	9	75
Rapeseed and mustard	97.0	0.1	—	0.1	0.05	3	8	85
Til	97.0	0.1	—	0.1	—	3	9	80
Groundnut	96.0	None	—	None	—	4	9	70
Sunflower	98.0	None	—	None	—	2	9	60
Soybean	97.0	None	0.1	5 seeds/kg	—	3	12	70
Vegetables								
Peas	98.0	None	0.1	None	—	2	9	75
Cowpeas	98.0	None	0.1	None	—	2	9	75
Rajma	98.0	None	0.1	None	—	2	9	75
Tomato	98.0	0.05	—	None	—	2	8	70
Cauliflower	98.0	0.05	—	0.05	—	2	7	65
Bhindi	99.0	None	—	None	None	1	10	65
Watermelon and other cucurbits	99 0	0.05	—	None	None	1	7	60
Onion	98.0	0.05	—	0.10	—	2	8	70
Carrot	95.0	0.05	—	0.1	—	5	8	60
Chillies	98.0	0.05	—	0.05	—	2	8	60
Radish	98.0	0.05	—	0.1	—	2	6	70
Brinjal	98.0	None	—	None	—	2	8	76

*Diseased seed standard : 1. Bajra ; ergot sclerotia, 0.02% (by number)
2. Wheat ; nematode galls of tundu/ear cockle or seeds infected by Karnal bunt, 0.10% (by number)
3. Rice ; paddy bunt, 0.10% (by number)

**For hybrid and other varieties, respectively. It includes seeds of other distinguishable varieties.

***For single cross hybrids and other varieties, respectively.

gram the maximum permissible limit for seeds of other varieties is 0.1 and 0.5% for foundation and certified seeds, respectively. In the case of rapeseed and mustard, the maximum permissible quantity of objectionable weed seed is 0.05 and 0.1% for foundation and certified seeds, respectively.

Foundation seed crops is subjected to field inspections in the same manner as those for certified seed. However, the standards for isolation distance (Table 28.4) and those for field inspections (Tables 28.6 and 28.7) are much more stringent in the case of foundation seed than those for certified seed. The State Seed Certification Agency (SSCA) carries out the field inspections and seed tests for foundation seed and, if the standards are met with, it issues the appropriate tags for the same.

OPERATIONS ESSENTIAL TO A SEED INDUSTRY

The following operations/activities are essential to a successful seed industry : (1) breeding of new varieties, (2) seed multiplication, (3) seed processing, (4) seed certification, (5) seed storage, (6) marketing and distribution, and (7) publicity.

Breeding of New Varieties. Breeding of new improved varieties of various crops is the basis for a seed industry. Development of new crop varieties is the responsibility of ICAR and its institutes, and of Agricultural Universities (Chapter 29). The breeder seed produced by plant breeders is the source of all the other classes of seed.

Seed Multiplication. Quality seeds used for commercial crop production are obtained from breeder seed through a stepwise multiplication as follows : breeder seed→foundation seed→certified seed (used for raising commercial crops). The breeder seed is regularly regenerated from the pure seed maintained by the concerned breeder/institution through multiplication coupled with purification.

Seed multiplication is undertaken by State Government Farms, State Farms Corporation of India, Agricultural Universities and progressive and competent farmers. Recommended seed production practices must be followed to ensure the purity and high quality of the seeds.

Seed Processing. Seed obtained from the threshing floor is dried to the appropriate moisture level ; cleaned to remove inert matter, seeds of other crops and of weeds ; graded to separate oversized and under-sized seeds of the same crop, treated with disinfectants and/or protectants ; and finally distributed in bags of an appropriate size. This entire process is done with the help of machines in a seed processing plant. A seed grower may develop his own facility for seed processing or he may utilize the existing facilities with a government/private agency.

Seed Storage. Seed produced in a season can be used for crop production in the next crop season only. Till that time, it must be stored in a clean, dry storage space to protect it from damage due to storage pests and adverse weather. For a longer storage, seeds

must be stored in a room with low temperature and relative humidity ; the National Seed Project is developing such facilities for the storage of breeder seed at different centres in the country.

Generally, processed seed is stored in bags which are arranged in suitable lots to facilitate inspection during storage. The height of the bag lots should not be more than 2—2.5 m for crops like soybean, groundnut etc. having seeds that are more readily damaged, and 3-4 m for crops like rice, wheat, maize, etc. which have relatively less damage prone seed.

Seed Marketing And Distribution. A proper distribution of seed is an important operation and presents a major problem in India since most of the farmers are illiterate. Seed distribution is the primary responsibility of the state governments and is being done through its extension and agricultural development agencies, *e.g.*, development blocks, agriculture officers, officers for specific crops etc. Other agencies involved in seed distribution are, private traders, agriculture universities, cooperative societies etc. The health of the seed industry of a country depends, to a large extent, on the efficiency of its marketing wing.

Seed Certification. Seed certification is the responsibility of the Seed Certification Agency (SSCA) of the concerned state. In those states where a SSCA does not exist, National Seeds Corporation (NSC) discharges this function. SSCA undertakes certification of seeds on the written requests of seed growers. For this purpose, SSCA carries out field inspections and seed tests to ensure that the seed crop and the seed meet the rigid quality requirements prescribed for the concerned class of seed ; on being satisfied, it issues appropriate tags to the seed growers for affixing them to seed bags. The main objective of seed certification is to keep a rigid control on the quality of seeds offered for sale by various agencies.

Publicity. Both the farmers and the seed industry stand to gain from the popularisation of quality seeds of improved varieties. Seeds of new varieties may be publicised by inserting suitable advertisements in news papers, agricultural magazines, radio, T.V. etc. National Demonstration trials, minikit trials, *Kisan melas*, *Kisan goshthis* etc. serve as extremely powerful tools for the popularisation of new varieties and the benefits from their quality seed. In fact, the most farmers in the country need no more to be educated on the importance of quality seed ; they need to be provided with only the information on which varieties to use, and where to get their quality seed. It is more urgent to ensure that the simple farmers are not cheated in the name of quality seed.

SEED PRODUCTION AND PROCESSING

Production of high quality seed requires considerable technical skill and a number of rigid requirements must be fulfilled in order to ensure a high purity and germination of seed. Seed multiplication involves two separate steps : (1) seed production, and

(2) seed processing. Seed production requires improved cultural practices, efficient weed, disease and pest control, optimum irrigation and fertilizer inputs and some other specific operations. Therefore, only progressive farmers are given the responsibility for seed production since they have all the required inputs for raising a good seed crop. Seed processing is ordinarily done by State Seeds Corporations who have the facilities for large scale processing of seed. But a farmer may develop his own facilities for seed processing if he is producing seeds on a large scale.

Seed Production

Seed production, for convenience, may be dealt with in two subheads : (1) isolation, and (2) cultivation.

Isolation. The crop raised for seed production should be separated from other fields of the same crop species by a minimum distance, which varies from one crop to the other. This distance is known as *isolation distance*. Isolation is essential to prevent pollination from unwanted pollen in the case of cross-pollinated and often cross-pollinated species and to avoid mechanical mixture and chance cross-pollination in self-pollinated species. The isolation distance

Table 28.4. Minimum isolation distance for seed certification in some crops.

Crop	*Minimum isolation distance (m)*		*Isolation distance modified by*		
	Foundation seed	*certified seed*	*Area of seed field*	*Additional border rows of the pollinator parent*	*Differential planting dates*
Hybrid maize	400	200	Yes	Yes	Yes
Hybrid jowar					
(*a*) other jowar	300	200	No	No	Yes
(*b*) Johnson grass	400	400	No	No	Yes
Hybrid bajra	1000	200	No	No	Yes
Wheat	3	3			
Rice	3	3			
Cotton	50	30			
Soybean	3	3			
Rapeseed and mustard	400	200			
Groundnut	3	3			
Sesame	100	50			
Peas	20	10			
Cowpeas, sem, rajma	50	10			
Tomato	50	20			
Bhindi	400	200			
Chillies	400	100			
Potato	—	100			
Brinjal	200	100			
Carrot	1,000	400-800			
Cucurbits	800	400			
Radish and turnip	1,600	1,000			
Cauliflower	1,600	1,000			

varies from 3 m in self-pollinated crops like wheat, rice, etc. to 200 m in the case of maize, bajra and jowar to even 400 m in case of jowar when the isolation is from Johnson grass (*Sorghum halepense*) (Table 28.4). In some cases, *e.g.*, hybrid maize, the minimum isolation distance may be considerably reduced by planting border rows of the pollinator parent and by choosing a larger field for seed production. But in certain other crops, *e.g.*, jowar and bajra, such a modification is not permitted. Even in case of maize, the isolation distance cannot be modified if the seed colour of the seed parent is different from that of the contaminating maize, or if the contaminating field is planted with sweet corn or popcorn. In any case, it is necessary to plant a variable number of border rows of the male parent in case of hybrid seed production to ensure a sufficient supply of pollen on the edges of the field.

Table 28.5. Modification of the minimum isolation distance in hybrid maize (*Z. mays*) seed production by the area of the seed field and by border rows of the pollinator (male) parent.

Area (ha) of the seed field				*Minimum number of border rows of the male parent*
4	*8*	*12*	*16*	
Isolation distance (m) from other maize				
200	190	180	170	1
175	165	155	145	3
150	140	130	120	5
87.5	77.5	67.5	57.5	10
50	40	30	20	13

It may be pointed out that the minimum isolation distance required for a foundation seed crop is markedly greater than that for a certified seed crop in the case of all those crops where pollen contamination is feared ; only in the case of strict self-pollinators like, wheat, rice, soybean etc., the isolation requirements for the two classes of seeds are identical, *e.g.*, 3 m. In most cases, the isolation needed for foundation seed crop is twice as much, or even more, as that needed for certified seed crop.

Cultivation. Improved cultural practices and recommended levels of fertilizer and irrigation must be provided to raise a healthy crop. The objective is to harvest the maximum yield of a high quality seed. The following important points should be observed for raising a good seed crop.

Land Requirement. The land should be level, fertile and free from noxious weeds common to the crop. In some cases, like bajra and jowar, the field to be used for seed production should not have been used for growing the same crop in the previous year. If the same crop was grown in the previous year, the field should be irrigated 3 weeks before sowing and ploughed just before sowing. This is done to allow the seeds that might have dropped in the field from the previous crop to germinate, and to destroy the seedlings by ploughing.

Cultural Practices. Recommended improved cultural practices must be followed for raising a good seed crop. Recommended does of fertilizers and irrigation water must be applied for high yields of high quality seed. Poor cultural practices would give lower yields and seeds of smaller size which would be rejected at the time of grading. This would drastically reduce the profits of seed growers.

Plant Protection. Adequate measures must be taken to protect the seed crop from diseases and pests. Insect pests and diseases may cause considerable damage to the crop reducing the yield and quality of seed. Further, incidence of some diseases may lead to rejection of the seed by the certifying agency as unfit for use as seed. For example, the maximum permitted incidence of head smut and grain smut in jowar (*S. bicolor*, seed parent only) is one head in 10,000 and one head in 2,500, respectively, at the time of last field inspection. This rigid requirement with respect to diseases is prescribed to prevent the occurrence of disease epidemics due to contaminated seed.

Weed control. Effective weed control is a must for good seed production. Weeds reduce crop yields and weed seeds contaminate the seed. Certain weeds are classified as objectionable weeds by the seed certification agency and the seed field is generally required to be free from such weeds. For example, in case of hybrid bajra, no objectionable weeds should be present at any field inspection ; if they are present, the seed field may be rejected.

Roguing. Roguing is the removal of plants which are off-type, that is, phenotypically different from the plants of the variety under certification. It is an important aspect of seed production and is necessary to prevent outcrossing and mechanical mixture. The off-type plants are regularly removed from the field either by uprooting or by cutting at the ground level. The off-type plants may differ in plant height, leaf characters, flowering time, maturity etc.

Special Operations. Seed production in certain crops may require some special operations. For example, hybrid maize seed production requires detasseling of the female parent before they shed pollen. Further, in hybrid seed production, the rows of male parent are harvested before the rows of female parent to avoid mechanical mixture of the two.

Harvesting. Harvesting and threshing of a seed crop should be done with considerable care in order to prevent mechanical mixture from other crop seed or weed seeds. The threshing floor should be clean and preferably cemented to keep the contamination by inert matter to a minimum. Care should be taken during threshing so as to avoid damage to seeds.

Seed Processing

The seed obtained from the threshing floor is contaminated by inert matter, other crop seed and weed seed, is of different sizes, may have excess moisture and its germinability is not known. This seed

is processed, tested, treated and bagged before it is offered for sale. The various steps in seed processing are, (1) drying, (2) cleaning and grading, (3) testing, (4) treating, (5) bagging and labelling.

Drying. Seeds must be dried to an appropriate moisture level (Table 28.1) in order to facilitate processing, to prevent losses in germination and to reduce the chances of insect attacks during storage. Drying generally involves the removal of moisture distributed inside the whole seed. This moisture first reaches the surface by capillary action from where it evaporates. Thus drying of seeds is a relatively slow process. Drying of seeds from rabi crops generally presents no problems since the weather at the time of harvesting is hot and dry. But in the case of kharif crops, and in some years in the case of rab crops as well, seed drying may become a major problem due to unfavourable weather conditions at the time of harvest.

Seeds may be dried (1) naturally or (2) artificially. *Natural drying* is done by spreading the seeds on trays, floors or fields in the open sun. Air movement and heat generated by the sun rays dry the seeds provided weather conditions are favourable. In case of unfavourable weather conditions, drying must be done artifically, *Artificial drying* involves passing of heated or unheated air through the seeds to remove moisture.

In *unheated drying*, normal atmospheric air is passed through the seed. For effective drying, the air should have low relative humidity ; therefore, it is not effective in a moist weather. It is a slow process and a larger drying space is needed. Slow drying may lead to the growth of moulds and thereby cause damage to seeds. It has the advantage of requiring low initial cost, no expense on fuel and there is no danger of fire.

Heated air method involves passing of heated air through the seed. This method is quicker, faster end requires less drying space than the unheated air method. Drying is not affected by weather conditions as is the case with the unheated air method. The disadvantages of this method are : high initial equipment cost, cost of fuel, danger of fire and possibility of overheating the seed which may reduce germination.

Cleaning And Grading. The seed from threshing floor is mixed with seeds of other crops and of weeds, pieces of straw, gravel, soil etc. Further, the seed is not of uniform size, but contains seeds of several sizes some of which are undersized, shrivelled and unfit for use as seed. *Separation of inert matter, weed seed and seeds of other crops from the seed is known as cleaning.* Seeds from different crops differ in size, shape, weight, specific gravity, surface smoothness, colour, electrical properties, stickiness etc. Machines are available that separate seeds on the basis of one or the other of the characteristics listed above. For example, *specific gravity separators* divide seeds on the basis of their weight and size ; *pneumatic separators*

separate seeds on the basis of their resistance to air-flow, *spiral separators* separate them on the basis of seed shape ; *velvet-roll separators* on the basis of surface smoothness ; *electronic separators* on the basis of electrical properties of seeds and *electronic colour-separators* on the basis of seed colour.

Grading is the removal of smaller and shrivelled seeds from the well filled healthy seeds. In India, *air and screen machine* is extensively used for cleaning and grading of seed. This machine uses air current for separating seeds on the basis of their resistance to air stream and uses sieves to separate seeds on the basis of their size and shape. Commonly, the air and screen machine has either two or three screens ; the size of the screens varies depending upon the crop. Dried seed is passed through the air and screen machine for cleaning and grading.

Testing. After cleaning and grading, the seed lots are tested for percentage of pure seed, weed seeds, seeds of other crops, inert matter and germination. This is known as *seed testing* and is done in a seed testing laboratory. Seed testing is an integral part of every seed certification programme and is used as a check on the quality of the seed to be marketed. The seed certification agency carries out seed tests on the seed lots presented for certification. We shall discuss in some detail the process of seed testing a little later.

Treating. Before bagging the seeds are treated with a suitable fungicide, often in combination with an insecticide. Seed treatment is helpful in the following ways : (1) it is helpful in controlling seed-born diseases, such as bunt in wheat, grain smut in jowar, seedling blight in maize, rice, jowar and wheat, *Fusarium* wilt of jowar and wheat etc., (2) it protects seeds from seed and seedling rots caused by *Phythium* and *Rhizoctonia* commonly present in soil, (3) protects against damage by storage pests, and (4) protects from damage by soil insects in the field.

There are two classes of seed treatment chemicals : disinfectants and protectants. *Disinfectants* inactivate the organisms present on the seed surface, *e.g.*, organo-mercurial compounds, like Agroson GN, Cereson etc. *Protectants* protect seeds from the attack by seed-born and soil-born pathogens, *e.g.*, Thiram, Captan, Chloranil etc. Organomercurials are injurious to seeds, particularly to cracked seeds. Therefore, they are used when disease incidence in the seed crop necessitates it and in crops where seed is not easily injured, *e.g.*, in rice, wheat, barley, cotton etc. Organomercurials are not used in such crops as maize, bean (*P. vulgaris*). peanut (*A. hypogaea*) and vegetable seeds. An appropriate insecticide is often combined with a fungicide to protect the seed from insect damage. The chemicals may be applied as *dry powder* or in the form of *slurry* ; the latter provides a more uniform coverage and is commonly used. The recommended schedule for seed treatment of some crops are summarised in Table 28.6.

Table 28.6. A summary of the recommended schedules for seed treatment.

Crop	*Chemical and its formulation (fungicide)*	*Nature of treatment*	*Quantity per 100 kg seed*	
			Chemical (g)	*Water in case of slurry (l)*
	Cereals And Millets			
Maize	Thiram 75% WDP	S	70	0.5
	or Captan 75% WDP	S	70	—
Rice	EMC or MEMC or	W	60	Sufficient to completely immerse the seeds
	Organo-mercurial 1%	D	250	—
Bajra	Thiram 75% WDP	S	75	0.5
	or Thiram 75% dust	D	300	—
	or Captan 75% dust	D	300	—
	Organo-merourial 1%*	D	250	—
	Brine** (salt solution)			Solution sufficient to completely immerse seeds
Jowar	Thiram 75% WDP	S	85	0.5
	or Captafol	S	200	0.5
Wheat	Thiram 75% WDP	S	100	0.5
	or Mancozeb	S	200	0.5
	Organo-mercurial 1%	D	250	—
	Carboxin***	S	250	0.5
	or Carbendazim***	D	250	—
	Pulses			
Gram	Captafol	S	250	0.5
Urd	Thiram 75% dust	D	250	—
	or Carbendazim 50% WP	S	100	0·5
Cowpea	Captan 75% WDP	S	100	0·5
	or Thiram 75% dust	D	250	—
Mung	Thiram 75% WDP	S	75	—
	or Captafol	D	250	—
	Carbendzaim	D	100	—
Arhar	Thiram 75% WDP	S	75	0.5
	Oilseeds			
Groundnut	Captan 75% dust	D	250	—
	or Thiram 75% WDP	S	125	0.5
	Captafol	D	200	—
Rapeseed and mustard	PMA 1% dust	D	250	—
	or Thiram 75% dust	D	150	—
Soybean	Captan 75% dust+	D	150	—
	Thiram 75% dust	D	150	—
	or Mancozeb	D	300	—
	or Captafol dust	D	300	—
	Fibres			
Cotton	Captan 75% dust	D	250	—
	or Thiram 75% WDP	S	100	0.5
	EMC, MEMC			Immerse the seeds for 6 hrs. in a 0.2% solution

Vegetables

Bhindi	Thiram 75% WDP	S	100	0.5
	or Captan 75% dust	D	250	—
Brinjal	Thiram 75% dust	D	250	—
	or Captan 75% dust	D	250	—
Carrot	Thiram 75% dust	D	250	—
Cauliflower etc.	Thiram 75% dust	D	250	—
Chillies	Thiram 75% dust	D	250	—
	or Captan 75% dust	D	250	—
Cucurbits	Thiram 75% dust	D	250	—
	or Capton 75% dust	D	250	—
Onion and tomato	Thiram 75% dust	D	250	—
Peas	Captan 75% WDP	S	100	0.5
	or Thiram 75% WDP	D	200	—

Note. 1. For all seeds, add DDT 50% WP to the weighed quantity of fungicide at the rate of 100 g DDT per kg of the fungicide, mix them well, and treat the seed with this mixture.

2. D, S and W denote dry dressing, slurry dressing, and wet treatment, respectively.

3. Where options are given, the chemicals (fungicides) are listed in the order of preference.

4. EMC, ethyl mercury chloride ; MEMC, methoxy ethyl mercury chloride ; PMA, phenyl mercury acetate.

5. *denotes that the seeds may be treated only if the grain smut infection in the field was more than the standard prescribed for certification.

6. **denotes that seed may be treated only if ergot infection in the field was present within the prescribed limits for certification ; fields having more infection than the limit are rejected.

7. Seed must not remain in brine solution for more than 5—10 minutes. After removal of ergot sclerotia, the seed must be repeatedly washed with clean water to remove all salt from the seeds.

8. ***denotes a specific treatment against loose smut. Only seed meant for raising a seed crop may be treated.

Bagging and Labelling. After seed treatment, seeds are distributed in bags of appropriate size (generally, 40 kg bags are used) ; the process is known as *bagging*. Each bag is labelled with an appropriate label (Fig. 28.2) which carries the following information : (1) kind of seed, (2) name of the variety, (3) purity, (4) per cent germination (5) date of germination test, (6) per cent weed seed, (7) per cent inert matter, (8) name and address of the seller, (9) period of validity of the certification, and (10) any other information pertinent to the seed.

Accurate labelling is important to the purchaser as it provides the necessary details about the seed. Seed laws require that accurate information is provided on the label. The Indian Seeds Act of 1966 is designed to regulate the quality of seed offerred for sale.

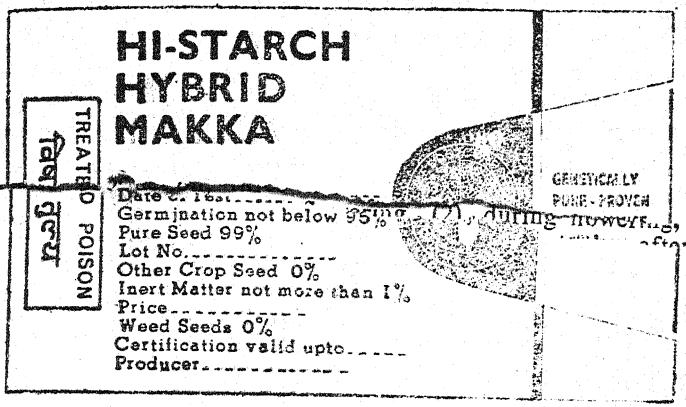


Fig. 28.3 Label for Hi-Starch Hybrid Maize used by NSC.

SEED CERTIFICATION

Seed certification procedures are designed to ensure physical and genetic purity, freedom from weed seeds and diseases and good germinability of the certified seed. Seed certification is done by the Seed Certification Agency (SSCA) of the concerned state or by NSC where such an agency does not exist. The process of seed certification is initiated by an application by the seed grower for certifying the seed produced by him. These applications are made on a prescribed proforma available from the concerned SSCA, and provide details about the location of farm ; area of the seed plot ; crop, variety and the class of seed proposed to be produced ; isolation etc. Generally, the application is made well in advance of the sowing of the seed crop, but in some cases it may be submitted even after the sowing of the crop. The SSCA scrutinises such applications and, on being satisfied, carries out field inspections and seed tests to ascertain the suitability of seed crops/seed lots for certification. If a seed crop/seed lot satisfies the prescribed purity and quality requirements, SSCA issues suitable tags of certification for affixing them to the seed bags under certification.

The decisions regarding the suitability of seeds for certification are based on the following inspections/operations : (1) field inspections, (2) inspection during seed processing and (3) seed tests (generally conducted on processed seed).

Field Inspection

Field inspections are done by seed certification inspectors from SSCA. The purpose of field inspections is to examine the seed crop in the field and to determine its suitability for certification. During field inspections, observations are made on isolation, the presence of off type plants, errors in planting, planting ratios in the case of hybrid varieties, presence of objectionable weeds and plants of other crops, and the incidence of diseases transmissible

through seed. There is a specified maximum for each of the above that is permissible during any field inspection ; the limit varies to

Table 28.7. The maximum permissible frequencies (as per cent of plants) of off type objectionable weed plants and plants infected by designated diseases in certified seed crop at any field inspection.

Crop	*off-type plants*	*Inseparable other-crop plants (%)*	*Objctionable weed plants*	*Plants infected by the designated diseases*	*Pollen producing plants of the seed parent*
Hybrid maize (other than single cross)	0.5*	—	—	—	1.0
Composite/ open-pollinated maize	1.0	—	—	—	—
Hybrid jowar	0.05*	—	—	0 1*	0.1
Jowar varieties	0.05	—	—	0.1	—
Hybrid bajra	0.05*	—	—	0.1**, 0.04**	0.1
Bajra OP varieties	1.0	—	—	0.1**, 0.04**	—
Rice, Wheat, Barley	0.3	0.05	0.02	0.5	—
Urd	0.2	—	—	—	—
Arhar and gram	0.2	—	—	—	—
Moong	0.2	—	—	0.2	—
Hybrid Cotton	0.2	—	—	—	—
Rajma	0 2	—	—	0.2	—
Pea	0.5	—	—	—	—
Cowpea	0.2	—	—	0.2	—
Rapeseed and mustard	0.5	—	0.1	0.2	—
Til	0.2	—	—	1.0	—
Groundnut	0.5	—	—	—	—
Soybean	0.2	—	—	—	—
Bhindi	0.2	—	None	0.2	—
Cauliflower etc.	0.5	—	—	0·5	—
Tomato	0.5	—	—	0.6	—
Brinjal	0 2	—	—	0 5	—
Carrot	0.2	—	—	—	—
Bhilli	0.2	—	—	0.5	—
Cucurbits	0 2	—	—	1.0+	—
Onion	0.5	—	—	—	—

*Both in the pollen and seed parents
**In the seed parent alone ; green-ear+green smut and ergot, respectively
***In the seed parent alone, ergot disease
+Only in muskmelon ; virus diseases.

some extent in different crops (Table 28.7). In case of cross- and often cross-pollinated crops, a very rigid check is applied on the presence of off-type plants during the various stages of flowering in order to prevent contamination through pollination. In each crop, a specific number of field inspections must be made at the specified stages of crop growth (Table 28.8). For example, in case of hybrid bajra seed production, three field inspections must be made at the following stages : (1) before flowering, (2) during flowering, preferably at or near full flowering, and (3) before harvest but after the seed is mature enough to reveal the seed characteristics.

Table 28.8. The minimum number of field inspections essential for seed certification in some crops

Crop	*Minimum number of field inspection*	*Stages of crop growth at which field inspection is done*
Hybrid maize, jowar and bajra	4	One inspection before flowering, two inspections during flowering, one inspection before or during harvesting.
Other varieties of maize	2	One inspection before flowering and one during flowering.
Jowar varieties	3	First inspection before flowering, second during flowering and third before or during harvesting.
Wheat, rice, barley, gram, arhar, pea, mung, urd, groundnut, soybean, cotton, cowpea, rajma	2	Anytime from the initiation of flowering till maturity.
Rapeseed, mustard, til, pea, cucurbits, tomato, brinjal, bhindi, chillies.	3	First inspection before flowering, second during flowering and third after fruits mature, before harvesting.
Carrot	3	First, 20–30 days after sowing, second, at the time of transplanting of roots ; and third, during flowering.
Onion (transplanted)	4	First, early stages of growth ; second, digging out of bulbs ; third, transplanting of bulbs ; and fourth, during flowering.
Potato	3	First, 45 days after planting ; second, before killing of aerial shoots ; third, after killing of aerial shoots.

Foundation seed crops are also subjected to the same number of field inspections as those for certified seed. However, the maximum permissible limits for definite off-type plants, doubtful off-type plants, objectionable weed plants, plants infected by specified diseases and pollen producing plants of the seed parent (in the case of hybrid varieties) in the case of foundation seed crops of various crop species are either one-half or less than those for their certified seed crops (Table 28.9). For example, the maximum permissible limits, in per cent, for plants of objectionable weeds in

rice are 0.01 and 0.02, for plants infected by specified diseases are 0.1 and 0.5, and for definite off-type plants are 0.05 and 0.30 for foundation and certified seed crops, respectively.

Table 28.9. The maximum permissible frequencies (% of plants) of, off-type plants, objectionable weed plants, and plants infected by designated diseases in a foundation seed crop at any field inspection.

Crop	*Definite off-type plants* (%)	*Inseparable other crop plants* (%)	*Objectionable weed plants* (%)	*Plants infected by designated diseases* (%)	*Pollen producing plants of the seed parent* (%)
Sigls cross hybrid maize	0.1 (both the parents)	—	—	—	0.50
Composite and open-pollinated maize	1.0	—	—	—	—
Hybrid jowar	0.01	—	—	0.05	0.05
Jowar varieties	0.01	—	—	0.05	—
Hybrid bajra	0.01	—	—	0.05, 0 02*	0.05
Bajra O.P. varieties	0.5	—	—	0.05, 0.02*	—
Rice, Wheat, Barley	0.05	0.01	0.01	0.1	—
Moong	0.1	—	—	0.1	—
Urd	0.1	—	—	—	—
Hybrid cotton	0.1	—	—	—	—
Gram	0.1	—	—	—	—
Arhar	0.1	—	—	—	—
Cowpeas	0.1	—	—	0.1	—
Rajma	0.1	—	—	0 1	—
Pea	0.1	—	—	—	—
Rapeseed and mustard	0.1	—	0.05	0.1	—
Til	0.1	—	—	0.5	—
Groundnut	0.1	—	—	—	—
Soybean	0.1	—	—	—	—
Bhindi	0.1	—	None	—	—
Cauliflower etc.	0.1	—	—	0.1	—
Tomato	0.1	—	—	0.1	—
Brinjal	0.1	—	—	0.1	—
Carrot	0.1	—	—	—	—
Chilli	0.1	—	—	0.1	—
Cucurbits	0.1	—	—	0.1**	—
Onion	0.2	—	—	—	—

*Green ear + green smut and ergot, respectively.

**Only in muskmelon ; virus diseases.

Table 27.10. A list of objectionable weeds and designated diseases for seed production of some crop species.

Crop	*Objective weeds*	*Designated diseases*
Wheat	*Convolvulus arvensis* (Hindi, *hirankhuri*)	Loose smut (*Ustilago tritici*) (Pers) Rostr
Jowar	Nil	Grain smut (*Sphacelotheca sorghi* (LK.) Clinton) Head smut (*Sphacelotheca reiliana*) (Kuehn) Clinton)
Bajra	Nil	Grain smut (*Tolyposporium penicillariae* Bref.) Green ear (*Sclerospora graminicola* (Sacch) Schr.) Ergot (*Claviceps microcephala* (Wall.) Tul.)
Toria and mustard	*Argemone mexicana* (Hindi, *satyanashi*)	*Alternaria* blight (*Alternaria brassicae* (Berk.) Sacc.)
Cowpea	Nil	Anthracnose (*Colletotrichum capsicae* (Syd) Butl. & Bisby *Ascochyta* blight *Ascochyta phaseolorum* Sacc.) Bacterial Blight (*Xanthomonas compestris* pv. *vignicola*)
Moong	Nil	Halo blight (*Pseudomonas syringae* pv. *phaseoli*)
Pea	Nil	Pea mosaic (*Pisum* virus 2A; Syn. pea virus 3)
Cucurbits	Wild cucurbits	Pumpkin mosaic (*Cucumis* virus)
Bhindi	Wild *Abelmoschus* species	Yellow mosaic (*Hibiscus* Virus 1)
Potato	Nil	Brown rot (*Pseudomonas solanacearum* E.F. Smith) Leaf roll (potato virus 1 ; Syn., *Solanum* virus 14). Mild mosaic (potato virus X ; Syn., *Solanum* virus 1). Root Knot nematode (*Meloidogyne* sp) Golden nematode of potato (*Globodera rostochiensis* Wr)* Scab (*Streptomyces scabies* (Thaxt) Wakoman & Hemrici) Severe mosaic (potato virus Y) Wart (*Synchytrium endobioticum* (Schilb) Perc.)

*It occurs in the Nilgiri Hills area ; movement of seed potato grown in this area to other areas of the country is strictly prohibited.

In case of some crops, certain weeds are classified as *noxious* or *objectionable weeds* (Table 28.10) because their seeds are similar to and very difficult to separate from those of the concerned crops,

they are difficult to eradicate once they infest a field and/or they may act as hosts for diseases or insect pests of the respective crops. Similarly, certain diseases of some crops are *designated* (Table 28.10) since their pathogen is transmitted through seed (propagule). Objectionable weed plants and plants affected by designated diseases are specifically monitored during field inspections.

Inspection During Seed Processing. The inspection during seed processing is done to determine if the seeds have been dried to the appropriate moisture level, and if the correct processing procedure is being followed. In case of maize, a check is also made to see that undesirable cobs have been separated and rejected before shelling. Another purpose of such inspections is to determine if appropriate care is taken to avoid mechanical mixtures during seed processing.

Seed Tests

Seed tests consist of a series of tests designed to determine the quality of seed. Seed tests are done in seed testing laboratories. Almost every state has a seed testing laboratory which performs the following functions.

1. Conducting research on seed testing methods.
2. Training of personnel in seed testing.
3. Determining the standards for seed purity and seed quality for various crops.
4. Seed testing for certification and for implementation of seed laws of the country. The latter implies that the seed tests conducted by the seed testing laboratories are accepted as evidence by the courts of law in India.

Before certification, the seed lots are subject to seed tests in seed testing laboratories. The seed has to meet a specified standard before it can be certified. The minimum standards for seed certification vary to some extent from one crop to the other (Table 28.1). Generally, the following tests are conducted to determine the quality of seeds : (1) purity test, (2) germination or seed viability test, and (3) moisture content test.

Sampling. Seed tests are conducted on small samples drawn generally from processed seed lots. It is essential that the samples used for seed tests are a representative of the lot. In other words, the results obtained from the samples should project the real picture of the seed lot from which they were drawn. Therefore, elaborate attempts are made to draw a random sample from seed lots. If the sample represents a single bag, samples should be drawn with the help of a trier from three or more places of the bag and, composited. If the seed sample represents a number of bags, one sample should be drawn from each bag or from a random selection of bags and the seed from such samples should be composited. The idea is to obtain a sample which represents the entire seed lot, and not one or

two bags of the seed lot. The composited seed is thoroughly mixed and is subdivided into a number of smaller samples, known as *working samples.* The weight of a working sample is usually 25 g. Often the seed samples are mixed and subdivided into working samples with the help of certain machines, *e.g.*, Boerner Divider, Gamet Precision Divider.

Purity Test. *Purity denotes the percentage of seeds (by weight) belonging to the variety under certification.* The working sample is closely examined, often with the help of a magnifying glass, to classify it into the following components.

1. *Pure seeds* ; seeds of the variety under certification
2. Seeds of other varieties of the same crop
3. Seeds of other crops
4. Seeds of weeds/objectionable weeds
5. *Inert matter* ; sand, straw, stones, pebbles, soil particles etc.
6. *Defective seeds* ; broken and shrunken seeds. A broken seed which is larger than half of the original size and has intact embryo is classified as pure seed. *The defective seeds are classified as inert matter.*

The components 2 to 6 are impurities ; there is a maximum permissible limit for each of these impurities in the seeds of different crops (Table 28.1). The purity of the seed is calculated on the weight basis as follows.

$$\text{Purity (as per cent)} = \frac{\text{Weight of pure seed}}{\text{Total weight of the working sample}} \times 100$$

$$= \frac{\text{Weight of pure seed}}{\text{Weights of (pure seed + seeds of other varieties + seeds of other crops + weed seeds + inert matter)}} \times 100$$

$$\text{Similarly, Impurity (\%)} = \frac{\text{Weights of (seeds of other varieties + seeds of other crops + weed seed + inert matter)}}{\text{Total weight of the sample}} \times 100$$

Impurity percentage is also referred to as *dockage.* The purity test is done on two or more samples from the same seed lot to make sure that the results are resonably reliable.

Separation of seeds of other varieties of the same crop from those of the variety under certification is often more difficult than that of other impurities, *e.g.*, other crop seed, weed seed etc. In many crops, *e.g.*, arhar, gram, rajma etc., seeds of different varieties may differ in colour, shape, size and or other *morphological characteristics* ; this permits a relatively easy separation of other varieties seeds. In case of wheat, *phenol reaction* of seeds is used as a basis for determining the frequency of seeds of

other wheat varieties. For this test, seeds are presoaked for 24 hr and placed on filter papers soaked in a 1% phenol solution (usually kept in petri plates). Usually, a 4 hr period is allowed for the seeds to develop colour ; sometimes a longer period may be allowed to permit a more accurate identification. Seeds of different varieties differ in their reaction to phenol, and this provides the basis for their identification. This technique may be used in barley also.

In some crops, *e.g.*, *Aneva*, *Lolium*, *Pisum* etc., seeds of other varieties are identified with the help of *ultra violet* (*UV*) *light*. Seedlings of many crops, *e.g.*, *Triticum*, *Gylcine*, *Sorghum*, maize, sugarbeet, moong etc., develop anthocyanin, and their varieties may differ for this characteristic. In such cases, seeds are germinated, generally in petri plates, and the seedlings are classified on the basis of their *anthocyanin pigmentation* (*colour*). Some special treatments may be used to intensify the anthocyanin pigmentation of seedlings, *e.g.*, germinating wheat seeds on filter papers soaked in 1% solution of sodium chloride, and exposing the seedlings to UV light for 1-2 hr before observation. *Other seedling characteristics*, *e.g* , hairiness, intensity of chlorophyll pigmentation etc. may also be used for identification of seeds of other varieties.

A *grow out test* may be resorted to for a more definite determination of seed purity. In such a test, at least 100 plants are grown with proper spacing and under optimum management either in a green house or in an off-season nursery. Various morphological featues of the plants, including flower colour and other floral features, plant height, days to flower, days to maturity, fruit and ear head features etc. are scrutinized in an effort to determine the frequency of other varieties seeds in a seed sample. A grow-out test is perhaps the most precise but is rarely used in this country as it requires a much longer time, an excellent greenhouse/off-season nursery facility, a much greater effort and funds than the tests based on seed and seedling characteristics. The test based on seed morphology is the most rapid and quite simple and the least expensive, but its applicability is quite limited. Any test for purity must be rapid, inexpensive and simple since a large number of seed samples have to be screneed for purity.

Seed Viability or Germination Test. *Germination is determined as per cent of seeds that produce or are likely to produce seedlings under a suitable environment.* Thus germination test is of great importance because the sole function of seed is to produce healthy seedlings for raising a good crop. The two tests most commonly used for the determination of seed viability are germination test and tetrazolium chloride test.

Germination Test. *Germination test determines the percentage of seeds that produce healthy root and shoot.* In most of the cases, seeds are germinated on wet filter papers placed in Petri dishes. The Petri dishes are kept under controlled conditions in an incubator or in a culture room. For most species, a temperature between

18-22°C is adequate ; for some species a specific temperature may be required. Generally, diffuse light or even darkness suffices. Where required, specific environment should be provided for germination tests. The duration of germination test varies from 7-28 days depending upon the crop species. For most of the cereals and many legumes, 7 days are enough, but in many grasses longer periods are required. Germinated seeds are counted at regular intervals and are removed from the Petri dishes. The total number of germinated seeds would be the sum of the number of seeds that germinated at different observations. The per cent germination is calculated as follows.

$$\text{Germination (\%)} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds plated}} \times 100$$

For convenience, 100 seeds are plated in each sample. From each seed lot, 4 or more samples are plated for a reliable germination estimate. If there is a difference of 10 per cent or more in the germination of different samples from the same seed lot, it is desirable to repeat the germination test.

Tetrazolium Method. *This method determines the percentage of viable seeds which may be expected to germinate.* The chemical 2, 3, 5-tetrazolium chloride, or tetrazolium chloride in short, is colourless, but it develops intense red colour when it is reduced by living cells. This phenomenon is used to determine the percentage of viable seeds in a seed sample. Seeds are soaked in tap water overnight and are split longitudinally with the help of a scalpel so that a portion of embryo is attached with each half of the seed. One half of each seed is a placed in a Petri dish and covered with 1% aqueous solution of tetrazolium chloride for 4 hours. The seeds are then washed in tap water and the number of seeds in which the embryo is stained red is determined. The per cent of viable seeds is computed as follows.

$$\text{Viable seeds (\%)} = \frac{\text{Number of half seeds stained red}}{\text{Total number of half seeds}} \times 100$$

The tetrazolium method is faster than the germination method and it does not require a controlled environment which is necessary for the germination test. It is relatively cheaper than germination test. But it cannot be applied to all the species, particularly to those species that have very small seeds and embryos, because splitting and examination of such seeds is tedius.

Real Value of Seed. *The real value of seed is the percentage of a seed sample that would produce seedlings of the variety under certification.* This is also known as *utility percentage* of the seed, and is a function of the purity and germination percentages of the seed sample. The real value of a seed lot is determined as follows.

$$\text{Real value of seed (\%)} = \frac{\text{Purity (\%)} \times \text{Germination (\%)}}{100}$$

Clearly, when two or more seed lots are being compared, their purity and germination should be taken into account. This is easily done by determining the real value of seed lots.

Moisture Content. *Moisture content is determined as per cent water content of the seeds.* Optimum moisture content reduces the deterioration during storage, prevents attack by moulds and insects and facilitates processing. The moisture content is determined by drying the seeds in an oven or with the help of a moisture metre. In the case of *oven method,* weighed seed samples are dried at 130°C for 90 minutes in an oven. The dried seeds are weighed again. The loss in weight represents the weight of water lost due to drying. The moisture content is estimated as follows.

$$\text{Moisture content}\,(\%) = \frac{W_1 - W_2}{W_1} \times 100$$

where W_1 is the weight of the seed sample before drying and W_2 is the weight of the seed sample after drying.

Moisture metres measure the resistance of seeds to an electrical current ; the electrical resistance of seeds varies with the moisture content. Use of moisture meters requires calibration and a certain degree of technical skill on the part of the user. But moisture meters are very efficient and extremely rapid ; a large number of samples can be handled in a relatively short period.

MAINTENANCE OF IMPROVED SEED

The improved seed sold to the farmers is certified seed. Certified seed may be the progeny of certified, registered or foundation seed. These seed classes are ultimately derived from breeder seed, which is maintained by the breeder or the institution that produced the variety. Every attempt is made to keep the breeder seed genetically pure and to retain the original genetic composition of the variety. The improved varieties are maintained as breeder seed ; the practices for the maintenance of breeder seed vary from one crop species to the other mainly depending on the mode of reproduction of the crop and the nature of the variety to be maintained.

Self-Pollinated Crops. Isolation (3 m) is provided to prevent outcrossing and care is taken to avoid mechanical mixtures. Off-types are promptly rogued out. Every year, mass selection is done to maintain genetic purity (Chapter 10). Several hundred spikes are selected and separate progeny rows are grown. Off-type progenies are eliminated ; the remaining progenies are bulked. This maintains the genetic purity of the varieties. The seed obtained in this way is known as *nucleus* or *basic seed* ; it is the source of breeder seed.

Inbred Lines. Hybrid or synthetic varieties are maintained as inbreds used in producing them. The inbreds are maintained by strict hand pollination. Off-type plants are promptly rogued to maintain genetic purity of inbreds.

Asexually Propagated Crops. Varieties of asexually propagated crops are maintained by asexual reproduction. Mechanical mixtures are avoided and off-type plants are promptly eliminated.

Every effort is made to maintain the genetic purity of varieties. Since the breeder is directly responsible for the maintenance and multiplication of the breeder seed, he is able to maintain the genetic purity of the varieties.

SEED PRODUCTION ORGANISATIONS

There are two types of government/public sector organisations responsible for seed production and certification in India. The first type of organisation is represented by the National Seeds Corporation (NSC) which has responsibilities for the entire country. The second type of organisations are State Seeds Corporations (SSCs) and State Seed Certification Agencies (SSCAs) that have statewise responsibilities. In addition, a number of private seed companies are also engaged in seed production activities, *e.g.*, Hindustan Lever Ltd., Bombay ; Pioneer Seed Company Ltd., Hyderabad ; Maharashtra Hybrid Seed Company Ltd., Jalna ; Indo-American Hybrid Seed, Bangalore etc. These companies deal exclusively with hybrid seed production of various crops, *e.g.*, cotton, maize, jowar, arhar etc.

National Seeds Corporation

The National Seeds Corporation (NSC) was initiated in 1961 under the Indian Council of Agricultural Research. Later, on 7 March 1963, it was registered as a limited company in the public sector. The NSC was established to serve two main objectives : *first*, to promote the development of a seed industry in India and *second*, to produce and supply the foundation seeds of various crops.

Before the establishment of NSC, there was no organised seed industry in India for any of the farm crops. The seeds were sold by private traders, often without any check on their genetie purity, physical purity or germination. With the development of hybrid maize, the necessity of an organisation for seed production was urgently felt. NSC was established to fulfil this need, and the production of hybrid maize seed was its first responsibility. Later on, it was given the responsibility of seed production of hybrid jowar, hybrid bajra, wheat, jute, vegetatively propagated crops, forage species, various vegetables and other crops. Some of the functions of NSC have now been delegated to the other two agencies: State Seeds Corporation and State Seed Certification Agency. NSC handles foundation and certified seeds of nearly 230 varieties of 70 crops. The present functions of NSC may be summarised as follows.

1. Production and supply of foundation seed.
2. To maintain improved seed stocks of improved varieties.

3. Interstate marketing of all classes of seed.
4. Export and import of seed.
5. Production of certified seed where required.
6. Planning the production of breeder seed in consultation with ICAR.
7. Providing technical assistance to seeds corporations and private agencies.
8. Coordinating certified seed production of several State Seeds Corporations.
9. Conducting biennial surveys of seed demand.
10. Coordinating market research and sales promotion efforts.
11. Providing training facilities for the staff participating in seed industry development.
12. Providing certification services to states lacking established and independent seed certification agencies.

State Seeds Corporations

The State Seeds Corporations are chiefly concerned with the production and supply of certified seed, and within the state marketing of certified seed. State Seeds Corporations have been recently established in order to reduce the workload of NSC. These corporations were established in view of the great success of and the impact made by the Tarai Development Corporation (TDC), Pantnagar (established on February 27, 1969), which had gained a virtual strangle hold on the seed market of U.P. almost to the exclusion of NSC. It is hoped that the State Seeds Corporations would be able to function more efficiently and would be able to stimulate a faster growth of the seed industry.

State Seed Certification Agency

The State Seed Certification Agency (SSCA) is responsible for seed certification in the concerned state. The SSCA makes field inspections and conducts seed tests required for seed certification.

The SSCA performs the following functions : (1) it screens the applications from seed growers for seed certification and decides on their fitness, (2) it also checks and verifies the appropriateness of the source seed used for growing the seed crop under certification, (3) it carries out the requisite field inspections, (4) it conducts the seed tests, (5) it certifies the seeds found suitable and issues the appropriate tags both for certified and foundation seeds, (6) it guides the seed growers on production, processing and distribution of seeds, (7) it conducts short courses on seed production etc. for seed growers, (8) and it participates in other activities conducive to the development of seed industry, *e g.*, preparing and publishing lists of plant breeders, seed growers etc.

Each state has a *State Seed Certification Board* which supervises the activities of SSCA. Persons involved in seed processing and distribution, including businessmen dealing with production, processing and marketing of seeds, and scientists from agricultural universities, are the members of this board. In addition, there is a *Central Seed Certification Board* which advises the state governments and their SSCA on the matters of seed certification. The chairman of the board is nominated by the central government. The members of the board are drawn from among the officials of the state departments of agriculture, scientists from the agriculture universities, and persons from the seed industry. The board may also appoint committees for specific tasks.

CERTIFIED SEED PRODUCTION IN SOME CROPS

Seed production practices vary to a great extent from one crop to another. The precautions necessary during seed production are largely affected by the natural mode of pollination in the crop species. In self-pollinated crops, fewer precautions are required than in cross-pollinated crops and in often cross-pollinated crops. We shall briefly examine the seed production practices in some important crop species. *viz.*, self-pollinated crops, hybrid maize, hybrid jowar, hybrid bajra and potato. *For certified seed production, only the varieties released by the Central or State Variety Release Committee and duly notified by the Ministry of Agriculture and Cooperation, Government of India, can be grown.*

Self-Pollinated Crops

Seed production in self-pollinated crops, such as rice, wheat, barley, linseed, most of the pulses and certain oilseeds, is fairly easy since there is very little outcrossing (less than 5%). The chief concern in seed production of these crops is to prevent mechanical mixtures. This is done by isolating the seed crop from other crops by a distance of 3 m, and by roguing the off-type plants from the seed crop. The cultivation of seed crop is essentially similar to that of the commercial crop. The various operations in seed production are briefly outlined below.

Land Requirements. Generally, there are no specific land requirements. The land should be suitable for the cultivation of the crop in question.

Isolation. Isolation of 3 m must be provided on each side of the seed field to prevent mechanical mixture and chance outcrossing.

Cultural Practices. For each crop, a standard package of cultural practices is available. For raising a good crop, the package of practices should be strictly followed. If the package of practices is not followed, there may be a reduction in yield and in seed size. Both these would lead to a lower income to the seed growers.

Plant Protection Recommended plant protection practices must be followed to protect the crop against the diseases and insect pests.

Seed-born diseases like loose smut of wheat must be controlled because the presence of diseased plants may lead to the rejection of seed crop.

Weeding. The seed crop is required to be free from weeds, particularly from objectionable weeds. The presence of objectionable weeds would lead to the rejection of seed crop.

Roguing. Off-type plants are promptly rogued and so are the plants of other crops. This is essential to avoid mechanical mixtures and chance outcrossing. There is a maximum permissible limit for the presence of off-type plants during any field inspection (Table 28.7). Diseased plants are removed and destroyed.

Hybrid Maize (*Z. mays*)

The seed producers are supplied with seeds of two single crosses ; one of them is to be used as female or seed parent and is tagged with a yellow label, and the other is to be used as the male or pollinator parent and is tagged with a red label. The female parent is supplied at the rate of 45,000 seeds per hectare and the male parent is supplied at the rate of 15,000 seeds per hectare. This is the foundation seed and is supplied at the rate of *thousand viable kernels* and not on the weight basis.

Isolation. The isolation distance is 200 m ; it can be modified by planting border rows of the pollinator parent (Table 28.5). But when the contaminating maize is sweet corn or popcorn or has distinct seed colour from that of the seed parent, the isolation distance cannot be modified. Different planting dates may be used to modify isolation distance provided there are no receptive silks in the seed parent at the time of pollen shed in the contaminating field. The isolation distance is also modified by the area of seed field.

Cultural Practices. A standard package of cultural practices is available which must be strictly followed for raising a good, healthy seed crop. Recommended doses of fertilizer must be applied and interculture operations must be done to remove weeds etc.

Planting. The recommended planting ratio is 2 pollinator rows to every 6 rows of the female parent. The male rows should be marked at both the ends with flag, peg or marker plants like sunnhemp etc. At least one border row of the pollinator parent should be provided on each side of the field to ensure pollination at the edges of the field. The spacing should be 75-90 cm between rows and 20-25 cm between plants.

Plant Protection. The maize crop has to be protected against insect pests, such as, stem borers, hairy caterpillars, army worms, grasshoppers, maize beetle and white ants. Plant protection measures are available for the control of these pests.

Detasselling. Removal of tassel, the male inflorescence, before it has shed pollen is known as *detasselling*. If the female parent is male

fertile, detasselling must be done to ensure that there is no self-pollination in the seed parent. Detasselling is done by holding the plant with left hand a little below the tassel, grasping the full tassel in the right hand and removing the tassel by a steady upward pull. The tassel is then thrown on the ground. In most cases, the tassel is removed one or two days after the tassel is first visible, but in some cases, it may be necessary to remove the tassels before they emerge, Care must be taken to remove the entire tassel. Detasselling is done regularly every day as long as tassels emerge on the female plants. Three field inspections would be made during the pollination period to determine the percentage of female plants that shed pollen and a very rigid standard is maintained in this regard (Table 28.4).

Roguing. Off type plants are promptly removed before they shed pollen. There is a rigid requirement with respect to the presence of off-type plants during field inspections (Table 28.7).

Harvesting. Male rows are harvested before the female rows and the produce is removed from the field in order to avoid mixture with the harvest from the female lines.

Drying. The seeds at the time of harvesting have 30-32% moisture. Before shelling, the cobs are dried in drying bins to reduce the seed moisture to 12 per cent. The grains are shelled, cleaned and graded before treating and bagging.

Hybrid Jowar (*S. bicolor*)

The seed producer is supplied with foundation seed of the female parent (7.5 kg per hectare) tagged with yellow label, and of the male parent (5 kg per hectare) tagged with red label. The female parent is generally male sterile.

Land Requirement. If jowar was grown in the previous season, the field should be irrigated at least 3 weeks before sowing and ploughed just before sowing to kill the germinating seeds present in the field. The field should not have plants of Johnson grass.

Isolation. The minimum isolation distance from grain or forage jowar is 200 m, and from Johnson grass (*S. halepense*) it is 400 m. This isolation distance cannot be modified by planting of additional border rows, but differential planting dates may be used to modify the isolation distance provided the adjacent field has no pollen shedding plants at the time the female parent is receptive.

Cultural Practices. The recommended package of cultural practices, fertilizer application, irrigation, interculture etc. should be followed to raise a good crop.

Planting. Two rows of the male parent should be planted to every four rows of the female parent. Each male row must be marked with flags, tags, pegs or marker plants. Four border rows of the pollinator parent must be planted all round the field to facilitate pollination at the end of the rows. Row to row distance is kept 75 cm, while plant to plant spacing is 7.5-10 cm.

Plant Protection. The crop must be protected from shoot fly, stem borers, spider mites, midge, ear bug and caterpillars. Control measures are available for effective control of these pests.

Roguing. Off-type plants must be removed immediately upon discovery. The field should be kept free of weeds and no objectionable weed should be present in the field. A rigid standard has to be met for certification (Table 28.7). Pollen shedding female plants must be promptly eliminated.

Field Inspection. There would be three field inspections as follows.

1. *First Inspection* before flowering to determine isolation, presence of off-type plants, planting ratios and errors in planting.
2. *Second inspection* during flowering to check pollinating rogues and male fertile female plants.
3. *Third inspection* prior to harvest and after the seed has matured to reveal its true characteristics.

Harvesting. Male rows are harvested before the female rows in order to avoid mechanical mixture.

Drying. The seeds may need artificial drying. The moisture content of the seeds should be reduced to 12 per cent.

Hybrid Bajra (*P. americamum*)

The seed producer is supplied with 2.5 kg of foundation seed of the female parent (tagged with yellow label), generally male sterile, and 1.2 kg seed of the male parent (tagged with red label).

Land Requirement. If bajra was grown in the previous season, the field must be irrigated at least 3 weeks before sowing and ploughed just before planting. This is done to induce the seeds present in the soil to germinate and then to kill them by ploughing.

Isolation. The minimum isolation distance is 200 m. This distance cannot be modified by planting additional border rows, but it can be modified by differential planting dates. However, the contaminating field should not shed pollen when the female parent has receptive stigmas.

Planting. The recommended ratio of male rows to female rows is 2 to 4. The male rows are marked for quick identification. At least 8 border rows of the male parent must be planted all round the field to facilitate pollination. Row to row distance is kept 60-70 cm and plant to plant distance is maintained at 20-22 cm.

Cultural Practices. The recommended package of cultural practices should be followed for raising a healthy seed crop. Recommended doses of fertilizer, irrigation and interculture should be provided.

Plant Protection. Generally, pest control may not be necessary. But sometimes, shoot flies, borers, leaf rollers, midges, caterpillars, grass hoppers and certain beetles may become a problem and control measures may be necessary.

Roguing. Off-type plants must be removed immediately when they are discovered. Roguing should be done before, during and after flowering. Pollen shedding female plants are eliminated promptly. Roguing is done at maturity to remove plants with distinct characteristics. Roguing in bajra is done either by uprooting the plants or by cutting them at the ground level to prevent regrowth. A rigid standard has to be maintained for seed certification (Table 28.7).

Field Inspection. Three field inspections are made as follows.

1. *First inspection* is done before flowering to determine isolation, presence of off-type plants and planting ratios.
2. *Second inspection* is done during flowering to determine pollen shedding female plants and off-type plants.
3. *Third inspection* is made prior to harvest when the seed is mature enough to reveal its true characteristics.

Harvesting. Male rows must be harvested before the female rows to avoid mechanical mixture.

Drying. Artificial drying may be necessary to reduce the seed moisture to 12 per cent.

Potato (*S. tuberosum*)

The chief objective of seed production in potato is to produce seeds free from viruses Y, A, X and S. These viruses are transmitted by aphids. Aphid population is low in hills during April to August, while it is low in the plains during October to early January. The aphid population in the plains starts building up in the middle of January and continues till April end.

Nucleus seed. Nucleus seed of potato is produced in the hills during April to August when the aphid population is low. This seed is brought to the plains and is stored in cold-storage for planting the seed crop in October.

Seed Production in Plains. In plains, the main potato crop is taken during November to March. But in some areas, *e.g.*, Jullundur (Punjab), two crops of potato are taken ; the first crop is taken from October to February, and the second crop is taken from January to May. The potato seed is multiplied in these areas during October to mid January. The potato seed is multiplied at Fagu, Jullundur, Daurala and Kodaikanal. The seed production practices in potato are briefly outlined below.

1. The crop is planted in the first week of October.
2. The field is not fertilized heavily ; the seeds are planted closely so that a large number of small, seed-sized tubers are produced. Roguing of diseased and off-type plants is done very rigidly.

3. By middle of December, irrigation is restricted and it is gradually stopped by the end of December so that the plants dry up by the middle of January. The plants may be killed by herbicide spray in the second week of January if they are still green. This is done to prevent the transmission of viruses to tubers from the aerial parts due to aphid attack.

4. The tubers are allowed to remain in the soil up to the end of February or early March. If the land is required for planting another crop, tubers may be removed and spread thinly in a dark place. This is necessary to allow thickening of the skin which is essential for the storage of tubers in a healthy state.

5. The tubers are harvested, graded and kept in cold storage to serve as seed for the next year's commercial crop. The seed for the main crop and for the second crop in the plains are obtained from the seed crop of the previous year. One hectare of seed crop produces enough seed for 10-15 hectares of commercial crop.

The system of seed production in potato is known as the *Seed Plot Technique. The seed plot technique essentially consists of multiplication in the plains of virus-free seed obtained from hills. This is achieved by growing the seed crop in the plains during the aphid-free period between October to mid January.* This seed is virus-free and serves as seed for the commercial crop of plains in the next year.

SUMMARY

Seed is the seed or any other propagating material used for raising a commercial crop. The improved seed has four classes : (1) breeder seed, (2) foundation seed, (3) registered seed, and (4) certified seed. The seed produced by the breeder who developed the variety, or by the institution where the variety was developed is the *breeder seed. Foundation seed* is the progeny of the breeder seed and is used to produce registered seed or certified seed. *Certified seed* is grown by various agencies and is certified for use as seed by the State Seed Certification Agency. The requirements of good seed are : (1) genetic purity, (2) physical purity, (3) good germination, (4) freedom from weed seeds, (5) freedom from diseases, and (6) an optimum moisture level. The minimum standards for certification vary to some extent from one crop to the other.

Production of certified seed is a rigid process. Recommended standards of isolation, land requirements, cultural practices, weed control, plant protection measures, roguing and harvesting must be met for approval of the crop as a seed crop. After harvest, the seed is processed. Seed processing involves (1) drying, (2) cleaning and grading, (3) testing, (4) treating, and (5) bagging and labelling. The seed must be dried to recommended level by natural drying, or by passing heated or room temperature air through the seeds. The seed is then cleaned mechanically to remove weed seeds, seeds of other crops and inert matter. At the same time, it is graded on the basis of seed size. The seed is then tested for purity and viability, and is treated with a suitable fungicide, and often with an insecticide. The treated seed is generally bagged in 40 kg bags and is suitably labelled.

Seed certification is done by State Seed Certification Agency on the basis of field inspection and seed tests. The seed test is done in a seed testing

laboratory and consists of the following operations : (1) purity test, (2) viability test, and (3) moisture content test. *Purity* of seed denotes the per cent of seed by weight that belongs to the variety under certification. *Seed viability* is based on germination or a chemical test, such as the tetrazolium chloride test. Viable seeds are those that germinate, or may be expected to germinate. The *real value of seed* is computed by multiplying the per cent purity of seed with its germination percentage and is expressed as percentage. Moisture content of the seed is estimated either by drying in an oven or by moisture metres.

Maintenance of improved seed is an exacting process. Varieties of self-pollinated crops are purelines and are maintained by roguing off-type plants and by taking care to avoid mechanical mixtures. In case of cross-pollinated and often cross-pollinated crops, inbreds are maintained by hand pollination ; open-pollinated and synthetic varieties are maintained by open-pollination in isolation. Asexually propagated crops are maintained as clones and off-types due to mechanical mixture, sexual reproduction or mutation are eliminated promptly.

Seed production practices recommended for self-pollinated crops, hybrid maize, hybrid jowar, hybrid bajra and potato are described in some detail.

QUESTIONS

1. Define the following : seed, dockage, real value of seed, physical purity, genetic purity, roguing.
2. Write short notes on the following : (*i*) registered seed, (*ii*) breeder seed, (*iii*) foundation seed, (*iv*) certified seed, (*v*) seed test, (*vi*) germination test, (*vii*) field inspection, (*viii*) seed certification, (*ix*) NSC, (*x*) SSC, (*xi*) isolation, (*xii*) labelling, (*xiii*) seed plot technique.
3. Discuss the requirements for seed certification with the help of suitable examples.
4. Briefly explain the various classes of seed. Describe in brief the procedure of certified seed production of hybrid bajra.
5. Define seed certification. List the various operations in certified seed production. Describe seed processing in some detail.
6. Briefly describe the practices of cultivation of crops for certified seed production with the help of suitable examples.
7. What is seed test ? Briefly describe the various seed tests for seed certification. Discuss the value of seed test in a seed certification programme.
8. Briefly describe the organisations for seed production in India. Explain the procedure of certified seed production in self-pollinated crops.
9. Explain the importance of the following : grading, drying, seed treatment, labelling, disease and pest control in a seed crop, germination test, genetic purity, isolation, sampling in seed test, seed certification, roguing, detasselling.

Suggested Further Reading

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CHAPTER 29

Organisations for Crop Improvement in India

The organisation of agricultural research in India has undergone many changes. It began with the creation of a Department of Revenue, Agriculture and Commerce in 1871 (chief function collection of statistics ; no research activity) and has developed into the present chain of research institutes and agriculture universities with a scientific manpower comparable to, if not larger than, any single country in the world. The early development of agricultural research was associated with the recurrence of famines ; a cruel reminder of the low priority accorded to agricultural research and development during the British period. The development of agricultural research gained some momentum after the first and the second world wars. After India became independent, much emphasis was laid on agricultural research which has brought rich dividends in terms of increased agricultural production and near self-sufficiency of the nation in agricultural commodities. Before we examine the present organisations for crop improvement, it would be relevant to consider the history of agricultural research and development in India.

HISTORY OF AGRICULTURAL RESEARCH IN INDIA

Establishment of Agriculture Departments and Agriculture Colleges

The beginnings of a primordial department of agriculture in India are as recent as April 27, 1871, when a Department of Revenue, Agriculture and Commerce was established. The chief function of the department remained revenue. There was no work on agricultural development, and the sole function of the department in this area was the collection of statistics. But this did mark a beginning, and a recognition, howsoever insignificant, of the agricultural sector by the government. The credit for this humble beginning goes to Lord Mayo, the fourth Viceroy of India, and to A.O. Hume, a civilian of the Bengal Civil Service and one of the founders of the Indian National Congress. Ironically, the department was established by Her Majesty's Government with a view to supply cotton to the hungry textile industries of Manchester, and not to feed the famine ravished India.

India faced a severe famine during 1877-1878. Based on the report of the famine commission, the government of India resolved to set up a Central Department of Agriculture controlled by the

Imperial Secretariat. Simultaneously, agriculture departments were to be set up in the provinces to look after agricultural enquiry, agricultural development and famine relief. These departments were set up in 1881, and directors were subsequently appointed in most of the provinces. But the chief duty of the agriculture departments in the centre as well as in the provinces remained famine relief.

In 1892, an Agricultural Chemist and an Assistant Chemist were appointed to look after research and teaching. This marked the first scientific staff in the Department of Revenue and Agriculture, as the department was then known. In 1901, an inspector general of agriculture was appointed to advise the Imperial and the Provincial Governments on agricultural matters. An Imperial Mycologist was appointed in the same year, and an Entomologist was appointed in 1903. During the years 1899-1900, India faced the most severe famine on record. Lord Curzon, the then Viceroy of India, was convinced that the Government of India must pay an urgent attention to agriculture. As a consequence, an Agricultural Research Institute (now IARI, New Delhi) was established in 1905 in Pusa, Bihar (then in the Province of Bengal). The agriculture departments in the provinces were expanded. The provinces were subdivided into a suitable number of 'circles' on the basis of regional differences in soil and climate. Each 'circle' had a research farm, and was placed in charge of a Deputy Director of Agriculture. Between 1901 and 1905, Agricultural Colleges were established or reorganised at Pune, Kanpur, Sabour, Nagpur, Lyallpur (now in Pakistan) and Coimbatore. It was visualised that the staff at these colleges would combine teaching and research to the best advantage. But due to the lack of scientific and technical manpower and finance, the primary function of these colleges remained teaching and training.

Establishment of The Imperial Council of Agricultural Research (The Present-Day ICAR)

As a result of a constitutional reform in 1919, agricultural development was made a state subject ; centre retained the central agencies and institutes of agricultural research and training. Thus there was no agency to coordinate the activities of the central institutions and those of the departments of agriculture in the provinces. This was emphasised by the Royal Commission on Agriculture appointed in 1926, and headed by Lord Linlithgow. The commission proposed that an Imperial Council of Agricultural Research should be set up to promote, guide and coordinate agricultural research throughout India. The Council was to act as a clearing house for research schemes and information, to provide research scholarships and to guide the research activities of the central and provincial departments of agriculture.

The proposal of the Royal Commission on Agriculture was examined and the Government of India, Department of Education, Health and Lands resolved on 23 May, 1929 to set up an Imperial

Council of Agricultural Research. The first president of ICAR was Khan Bahadur Sir Mohammed Habibullah ; Diwan Bahadur Sir Vijaya Raghhavacharya was its first Vice-President and Mr. S.A.Hydari was the first Secretary. The governing body of the council had 16 members.

The name of the council was changed from Imperial Council of Agricultural Research to Indian Council of Agricultural Research in March 1946. The decision to make this change was taken by the Governing body of ICAR in a meeting presided over by Sir Jogendra Singh.

The Commodity Committees

In addition to ICAR, there were several Central Commodity Committees that were concerned with research and development activities related to specific crops. These committees were started by the Ministry of Food and Agriculture and were semi-autonomous bodies. They were financed partly by the government and partly by the taxes collected on the export of the concerned commodities. Some commodity committees had their own research stations or institutes located in the main growing regions of the crops concerned (Table 29.1), while some others financed research schemes conducted by the State Departments of Agriculture, *e.g.*, Spices and Cashewnut Committee. The Indian Central Cotton Committee was the first commodity committee established in 1921 on the recommendation of the Indian Cotton Committee (1917-18). The functions of this Central Committee were cotton improvement, development of improved methods of growing, manufacturing and marketing of cotton. The committee financed schemes on cotton breeding, diseases, pests, physiology, agronomy etc. The committee was responsible for the development of 70 improved varieties of cotton, and the fiber quality of Indian cotton was considerably improved. In view of the success achieved in research on cotton under the Indian Central Cotton Committee, Commodity Committees were set up on other crops, *viz.*, lac, jute, sugarcane, tobacco, coconut, oilseeds, spices and cashewnut and arecanut (Table 29.1). As a result of this, the functions of ICAR became limited to food crops, tuber crops, grasses and fodder crops, horticultural crops, problems common to all the crops for which commodity committees were set up, *e.g.*, diseases, pests etc., dry farming and animal research.

The Vice-President of ICAR was the President of all the Commodity Committees. Apart from this link between various commodity committees, there was no other coordination in their activities. Further, the recearch stations for the various crops were located in the region where the crop was the most widely grown. But the soil and climate vary to a great deal from one region of the country to another, and there was a great necessity to conduct the researches on various crops within the different agroclimatic regions of the country. These realisations led to the formulation of the Project for

Table 29.1. The Indian Central Commodity Committees

Name of the Indian Central Commodity Committee	*Year established*	*Research institute/station*
Cotton Committee	1921	Technological Laboratory (now CTRL)
Lac Cess Committee	1931	Indian Lac Research Institute, Namkum, Ranchi (1936)
Jute Committee	1936	Jute Agricultural Research Institute, Barrackpore Jute Technological Research Laboratories, Calcutta
Sugarcane Committee	1944	Sugarcane Breeding Institute, Coimbatore Indian Institude of Sugarcane Research, Lucknow
Coconut Committee	1945	Central Coconut Research Station, Kanyagulam and Kasaragod
Tobacco Committee	1945	Central Tobacco Research Institute, Rajahmundry (8 substations)
Oilseeds Committee	1947	Financed research schemes ; headquarters at Hyderabad
Arecanut Committee	1949	Arecanut Research Station, Vittai (Karnataka)
Spices and Cashewnut Committee	1958	Financed research schemes

Intensification of Regional Research on Cotton, Oilseeds and Millets (PIRRCOM), which was the first step in the country towards coordinated approach to agricultural research.

The Central Commodity Committees were later abolished (beginning in 1965) and the research institutes under their control were transferred to ICAR.

Project for Intensification of Regional Research on Cotton, Oilseeds And Millets (PIRRCOM)

A need was felt to coordinate the research on various crops, *e.g.*, cotton, oilseeds and millets, and also to conduct the research work in different agroclimatic regions of the country. The first coordinated research work on regional basis was initiated in 1956 as a joint effort by ICAR and the Indian Central Committees on Oilseeds and Cotton. Seventeen centres were established throughout the country (Table 29.2) to conduct research on cotton, castor, groundnut, *Brassica*, til, toria, taramira, jowar and bajra. These research stations, except the one at IARI, were under the administrative control of ICAR. The research programme for each region was prepared by a regional coordination committee headed by the Agriculture Commissioner of India, and approved by the respective commodity committees. A regional station consisted of full-fledged sections of plant breeding and genetics, agronomy, agricultural chemistry and soil science, plant pathology and entomology.

Table 29.2. The Centres for the Project for Intensification of Research on Cotton, Oilseeds and Millets

Location of centre	*Province*	*Research work on*
Coimbatore	Tamil Nadu	Cotton, jowar, groundnut
Bellary	Karnataka	*Setaria*, rabi jowar
Dhadesagur		Cotton, Jowar
Dharwar		Kharif jowar
Sulakere		Ragi groundnut
Rajendrasagar	Andhra Pradesh	Castor, groundnut
Amravati	Maharashtra	Cotton, jowar, groundnut
Mohol		Rabi, jowar
Junagarh	Gujarat	Jowar, groundnut
Surat		Cotton, jowar
Gwalior	Madhya Pradesh	Kharif jowar
Hoshangabad		Linseed
Ajmer	Rajasthan	Jowar, bajra
Kanpur	Uttar Pradesh	Indian mustard, bajra
Patiala	Punjab	Toria, taramira
Sirsa	Haryana	Cotton
IARI	New Delhi	Cotton, jowar, bajra, linseed (fundamental research on physiology and cytogenetics ; linseed breeding)

Initiation of All India Coordinated Research Projects

The concept of coordinated projects first developed in relation to hybrid maize improvement. ICAR was interested in utilizing heterosis for maize improvement as this approach was highly successful in U.S.A. and several other countries. Rockefeller Foundation, then actively involved in crop improvement programmes in Mexico, Central America and the Caribbean, was invited to assist in the maize improvement programme in India. The Ministry of Food and Agriculture, Government of India, signed an agreement with the Rockefeller Foundation in 1956. According to this agreement, Rockefeller Foundation was to assist in the development of (1) the postgraduate school of Indian Agricultural Research Institute (IARI), New Delhi, and (2) research programmes on the improvement of some crops (maize, jowar and bajra, initially). Two scientists from the Rockefeller Foundation maize programmes in Mexico and Columbia came to India to study the position of maize crop and submitted their report. This report was scrutinised by the Botany Committee of ICAR and then by the Advisory Board of the Council, and provided the basis for the coordinated maize project.

In the coordinated maize improvement programme, the entire country was divided into major agroclimatic zones without any regard to state boundaries. Each zone was to have several research centres. Research centres present in a state would be under the

administrative control of that state. ICAR was to provide a Project Coordinator who would visit all the centres under the project to facilitate smooth running of the project. There would be annual workshops of the principal research workers to review the progress during the year and to formulate the research work for the next year. The Rockefeller Foundation agreed to provide the world collection of germplasm, and to provide another Project Coordinator in the early stages of the programme ; this coordinator was to work in close cooperation with the Project Coordinator appointed by the Council. With this set up, the All India Coordinated Maize Improvement Project was initiated in 1957. *This was a landmark in the agricultural research in India ; a coordinated approach for the whole country was initiated in place af the fragmentary and isolated research programmes in the past.*

The coordinated maize project proved to be the turning point in research planning in agriculture. By 1961, new high yielding maize hybrids became available as a result of the coordinated project. Spurred by this success of the concept, ICAR decided in 1965 to initiate coordinated projects on other crops as well as in other areas of research, *e.g.*, animal husbandry, soil sciences etc. Within 3 years of this decision, 70 coordinated projects on various subjects were launched, The coordinated projects accounted for 40 per cent of the total outlay for agriculture in the Fourth Five Year Plan. The progress of the coordinated projects was critically reviewed in the Fifth Five Year Plan ; some projects were terminated, some were merged with other projects, some projects were elevated to the level of Project Directorates and some were changed to Coordinated Programmes. In the Fifth Five Year Plan, there were 49 coordinated projects and some coordinated programmes. The 22 coordinated projects related to crop improvement are listed in Table 29.3.

Reorganisation of ICAR

In 1963, the Agricultural Review Team was appointed by the Ministry of Food and Agriculture to scrutinise the organisation of agricultural research in India. The team was headed by Dr. Marion W. Parker of USDA (United States Department of Agriculture). The team submitted its report in March, 1964. Based on the recommendations of the team, ICAR was reorganised in 1966. ICAR was made a fully autonomous organisation. Various research organisations under the Department of Food and Agriculture and under the Central Commodity Committees were brought under the control of ICAR. The Governing Body of ICAR was reorganised to make it primarily a body of scientists and agriculturists. IARI, National Dairy Research Institute and Indian Veterinary Research Institute were made national institutes. A provision was made for the recruitment of scientists through selection committees of ICAR. It was made a policy that an agricultural scientist would be appointed as the chief executive of ICAR with the designation of Director General. In May 1965, Dr. B.P. Pal was appointed as the first

Table 29.3. All India coordinated crop improvement projects

Name of project	*Headquarters*	*Remarks*
	Project Directorates	
Wheat	New Delhi	IARI Campus
Rice	Hyderabad	Under CRRI, Cuttack
Pulses	Kanpur	IARI Regional Research Station, Kanpur
Oilseeds	Hyderabad	Project Coordinators : Groundnut, Rapeseed and mustard, Sesamum and niger, Castor, and Sunflower
	Coordinated Projects	
(*a*) **Food Crops**		
Maize	New Delhi	IARI Campus, first coordinated project, started in 1957
Jowar	Hyderabad	IARI Regional Research Station, Hyderabad
Barley	Karnal	IARI Regional Research Station, Karnal
Millets	Pune	MPKV, Rahuri, Pune Campus
Forage crops	Jhansi	IFGRI, Jhansi
National seed project	New Delhi	ICAR headquarters
(*b*) **Commercial Crops**		
Sugarcane	Lucknow	IISR, Lucknow
Sugarbeet	Pantnagar	G.B.P.U.A. & T., Pantnagar
Cotton	Coimbatore	Started in 1967
Jute and allied fibres	Barrackpore	JARI, Barrackpore, W.B.
Soybean	Indore	National Research Centre for soybean, Indore
Tobacco	Anand (Gujarat)	Started in 1970
Cotton project (assisted by World Bank	Nagpur	Started in 1976, Located at CICR, Nagpur
(*c*) **Horticultural Crops**		
Fruits	Bangalore	IIHR, Bangalore
Citrus	Bangalore	IIHR, Bangalore
Tuber crops	Dholi (Bihar)	RAU, Dholi Campus
Potato	Simla	Started in 1971, under CPRI, Simla
Vegetables	New Delhi	IARI Campus
Medicinal and aromatic plants	New Delhi	NBPGR, IARI campus
Spices and cashewnut	Kasaragod	Under CPCRI, Kasaragod
Coconut and arecanut	Kasaragod	Under CPCRI, Kasaragod
Under-utilized and under-exploited plants	New Delhi	NBPGR, IARI campus

Director General of ICAR ; he was simultaneously Vice-President of the Council. Four posts of Deputy Director General were created to assist the Director General.

At present, the total number of institutes under the Council is 33 ; those related to crop production and related subjects are listed in Table 29.4.

Table 29.4. Central institutes engaged in research work on the various aspects of crop production.

Institute	*Location*	*Year established*	*Remarks*
Indian Agricultural Research Institute (IAR I)	New Delhi	1905 (shifted to Delhi in 1936 from Bihar)	Teaching and Research ; 14 substations
Central Arid Zone Research Institute (CAZRI)	Jodhpur (Rajasthan)	1959	Research on aridity in arid and semi-arid regions
Cotton Technological Research Laboratory (CTRL)	Matunga (Bombay, Maharashtra)	1924 (transferred to ICAR in 1966)	Evaluation of quality of new strains ; 9 regional units
Indian Grassland and Fodder Research Institute (IGFRI)	Jhansi (U.P.)	1962 (transferred to ICAR in 1966)	Research on fodder and forage crops
Indian Institute of Horticultural Research (IIHR)	Hessaraghatta (Bangalore, Karnataka)	1967	Horticultural crops ; 3 stations
Jute Agriculture Research Institute (JARI)	Barrackpore (W.B.)	1953 (est. in Dacca in 1939, under ICAR since 1966)	Jute, mesta, ramie, sisal and sunnhemp
Jute Technological Research Laboratories (JTRL)	Calcutta (W.B.)	1938 (under ICAR since 1965)	Technological research on jute and other fibres
Indian Lac Research Institute (ILRI)	Namkum (near Ranchi, Bihar)	1925 (under ICAR since 1966)	Research on Lac cultivation and processing
Central Plantation Crops Research Institute (CPCRI)	Kasaragod (Kerala)	1970	5 regional stations, 7 research centres, 2 seed farms ; plantation crops and tree spices
Central Potato Research Institute (CPRI)	Simla (H.P.)	1949 (est. at Patna, shifted to Simla in 1956)	Potato ; 7 regional stations, 4 seed multiplication centres
Central Rice Research Institute (CRRI)	Cuttack (Orissa)	1945 (under ICAR since 1966)	Rice ; 4 substations

Institute	*Location*	*Year established*	*Remarks*
Central Soil Salinity Research Institute (CSSRI)	Karnal (Haryana)	1969	Management of alkaline soils ; 1 substation (a breeding section)
Indian Institute of Sugarcane Research (IISR)	Lucknow (U.P.)	1952 (under ICAR since 1969)	Sugarcane (breeding included)
Sugarcane Breeding Institute (SBI)	Coimbatore (Tamil Nadu)	1912 (under ICAR since 1969)	Sugarcane ; 2 substations
Central Tobacco Research Institute (CTRI)	Rajahmundry (Andhra Pradesh)	1947 (under ICAR since 1965)	Tobacco ; 6 stations
Central Tuber Crops Research Institute (CTCRI)	Trivandrum (Kerala)	1963	Tuber crops other than potato
Central Institute for Cotton Research (CICR)	Nagpur (Maharashtra)	1976	Cotton
Central Soil and Water Conservation and Training Institute (CSWCTI)	Dehradun (U.P.)	1974	Research/training on soil and water conservation
Central Institute of Agricultural Engineering (CIAE)	Bhopal (M.P.)	1976	Agricultural and related equipments
Vivekananda Parvatiya Anusandhan Shala (VPAS)	Almora (U.P.)	1924 (shifted to Almora in 1936 ; under ICAR since 1976)	Agricultural research for hills
National Bureau of Plant Genetic Resources (NBPGR)	IARI campus (New Delhi)	1976	Introduction and export of germplasm
National Bureau of Soil Survey and Land Use Planning	Nagpur (Maharashtra)	1976	Detached from IARI
ICAR Research Complex for North-Eastern Hills Region (RCNEHR)	Shillong (Meghalaya)	1975	Agricultural and animal sciences and fisheries ; 9 stations
Central Agricultural Research Institute for Andaman and Nicobar group of Islands CARI (ANGI)]	Port Blair	1978	Agricultural and animal sciences and fisheries

Institute	*Location*	*Years established*	*Remarks*
National Academy of Agricultural Research Management (NAARM)	Hyderabad (A.P.)		Training IARS recruits ; previously Central Staff College for Agriculture
Indian Agricul-t ural Statistics Research Institute (IASRI)	New Delhi	1959	Agricultural statistics

In June, 1972, the Government of India appointed a committee to review the recruitment and the personnel policies of ICAR and its institutes, and to suggest measures for their improvement. The committee was headed by Mr. Gajendragadkar, retired Chief Justice of India, and submitted its report in January, 1973. In view of the recomendations by this committee, a Department of Agricultural Research and Education was created in the Ministry of Food and Agriculture in December, 1973. The Director General, ICAR, was made secretary to the new department. The Minister of Agriculture was designated as the President of the council, while the Director General, ICAR, was made the Chairman of the Governing Body. The Advisory Board and the Standing Committee were abolished ; the functions of the Standing Committee were assigned to Scientific Panels. The Scientific panels for different disciplines consider and evaluate the suitability for financial assistance of research schemes. An Agricultural Research Service was initiated for the recruitment of scientific personnel under the Agricultural Scientists' Recruitment Board. A scheme for internal assessment and promotion was initiated. The credit for these changes goes to Dr. M.S. Swaminathan, the then Director General of ICAR.

The entire country was divided into 8 agroecological zones. For each zone, regional committees were set up ; the Director General of ICAR was made ex-officio chairman of these committees. The function of these regional committees is to review the status of agricultural research and education in the concerned regions. The Governing Body of ICAR is assisted by a Norms and Accreditation Committee, which looks after the development of Agricultural Universities and the grant of fellowships.

Development of Agricultural Universities

Before independence, higher education in agriculture was virtually neglected. In 1948, there were 17 agricultural colleges in the country, which were under the control of Director, Department of Agriculture of the respective states. Colleges for animal husbandry were separate from those for agriculture, and were governed by the Director, Animal Husbandry of the states. Research and extension were the responsibility of the agriculture and the animal husbandry departments of the states. The organisation, staffing pattern, pay scales of teachers and financial support (which was

solely by the state through the respective departments) were not suitable for a first grade education and training in agriculture.

The University Education Commission (1948-49) headed by Dr. S. Radhakrishnan, recommended that rural universities should be established. In 1950, Major H.S. Singh and Mr. A.N. Jha (Chief Secretary and Development Commissioner, U.P.) visited the Land-Grant Universities of the United States. They advised the then Chief Minister of U.P., Pandit Govind Ballabh Pant, to set up such a university, and he accepted their advice. This event may be regarded as the one which led to the initiation of agricultural universities. In 1955, the first Joint Indo-American Team was set up which recommended the establishment of rural universities in each of the states. The team felt that U.P. (Tarai), West Bengal (Haringhatta), Bihar (Patna), Orissa (Bhubaneshwar), Travancore-Cochin and Bombay (Anand) states were suitable for starting such universities.

Dr.H.W. Hannah prepared a blue-print for agricultural universities in 1956 ; this provided the basis for the proposal by the Government of U.P. to the Central Government (in September, 1956) for starting an agricultural university near Rudrapur in the tarai region of U.P. The Central Government agreed to the proposal on an experimental basis. The second Joint Indo-American Team was set up in 1959, which submitted its report in 1960. The team recommended that the Agricultural Universities should be autonomous ; should consist of colleges of agriculture, veterinary, animal husbandry, home science, technology and basic sciences ; should have inter-disciplinary teaching programmes ; and should integrate teaching research and extension. By 1961, there were demands from many states for agricultural universities and the Government of India accepted the organisation of a few more agricultural universities during the Third Five Year Plan.

In 1960, the Government of India appointed a committee for providing a model for the necessary legislation by the states for the establishment of agricultural universities. The committee was headed by Dr. R.W. Cummings and submitted its report in 1962. On the basis of this report, ICAR prepared the model act for the development of agricultural universities. During 1960-65, the fourth Five Year Plan, seven agricultural universities were established in U.P., Orissa, Rajasthan, Punjab, Andhra Pradesh, Madhya Pradesh and Karnataka. The United States Agency for International Development (USAID) contributed significantly to the development of agricultural universities through the Land-Grant Universities of U.S.A. This assistance was in the form of training of Indian scientists in U.S.A., stationing of U.S. scientists for teaching and research in Indian agriculture universities and a limited amount of equipments for teaching and research.

The Education Commission (1964-66), headed by Dr. D.S. Kothari, recommended that all aspects of agricultural research should

be the function of agriculture universities. Subsequently, the responsibility for research was delegated from State Departments of Agriculture to agricultural universities, but this change was not uniformly implemented in every state. The Review Committee on Agriculture Universities (1977-78), headed by Dr. M.S. Randhawa, made many useful recommendations for the development of agriculture universities. It noted that the quality of leadership and the financial support from the state were crucial factors in the development of agricultural univerisities. The committee suggested, among other things, that the Director General, ICAR, and Chairman, University Grants Commission, should be members of the selection committee for appointing the Vice-Chancellors for agriculture universities. The Model Act should be followed faithfully, and states should accept the responsibility for developing agriculture universities.

One of the original objectives of the ICAR was to undertake, aid, promote and coordinate agricultural education in the country, But this was not put into effective practice until the reorganisation of ICAR in 1966. A full-fledged Division of Agricultural Education was set up within the ICAR to fulfil this objective. The ICAR has been crucial in the reorgonisation of agricultural education in the country by providing the necessary guidance, financial aid (Rs. 41 crores during 1974-75 to 1978-79), and schemes for improving the quality of teaching and research, *e.g.*, centres of excellence, higher education in new areas, Professors of Eminence, faculty improvement, scholarships and fellowships.

The total number of agriculture universities is now 27 (Table 29.5). The agriculture universities have contributed to a great extent to agricultural education, research and development in the country. Many improved varieties have been developed in the agriculture universities and they have made numerous other contributions to some of which we shall return later.

Table 29.5. Agriculture Universities in India.

University	*Location*	*Year established*	*Remarks*
Govind Ballabh Plant University of Agriculture and Technology	Pantnagar (U.P.)	1960	First agriculture university ; single campus
Rajasthan Agriculture University (bifurcated from University of Udaipur)	Bikaner (Rajasthan)	1986	3 campuses
Orissa University of Agriculture and Technology	Bhubaneshwar (Orissa)	1962	Single campus
Punjab Agricultural University	Ludhiana	1963	Single campus
Jawaharlal Nehru Krishi Vishwa Vidyalaya	Jabalpur (M.P.)	1964	9 campuses
Andhra Pradesh Agricultural University	Hyderabad	1965	6 campuses
University of Agricultural Sciences	Bangalore (Karnataka)	1965	4 campuses

University	*Location*	*Year established*	*Remarks*
Mahatma Phule Krishi Vidyapeeth	Rahuri (Maharashtra)	1965	4 campuses
Punjabrao Krishi Vidapeeth	Akola (Maharashtra)	1969	7 campuses
Assam Agricultural University	Jorhat (Assam)	1969	Single campus
Haryana Agricultural University	Hissar (Haryana)	1970	Bifurcated from PAU
Marathwada Agricultural University	Parbhani (Maharashtra)	1972	Single campus
Rajendra Agricultural University	Pusa (Bihar)	1971	5 campuses
Konkan Krishi Vidyapeeth	Dapoli (Maharashtra)	1972	4 campuses
Tamil Nadu Agricultural University	Coimbatore (T.N.)	1972	3 campuses
Kerala Agricultural University	Vellanikkara (Kerala)	1972	3 campuses
Gujarat Agricultural University	Dantiwada (Dist. Bankantha, Gujarat)	1972	4 campuses
Vidhan Chandra Krishi Vishwa Vidyalaya	Haringhatta (Dist. Nadia, W.B.)	1974	
Chandra Shekhar Azad University of Agriculture and Technology	Kanpur (U.P.)	1975	2 campuses
Narendra Dev University of Agriculture and Technology	Kumarganj (near Faizabad, U.P.)	1976	Single campus
Himachal Pradesh Krishi Vishwa Vidyalaya	Palampur (H.P.)	1978	2 campuses
Institute of Agricultural Sciences	Varanasi (U.P.)	1980	Within the Banaras Hindu University
Birsa Agriculture University	Ranchi (Bihar)	1980	Single campus
Sher-e-Kashmir University of Agricultural Sciences and Technology	Srinagar (J & K)	1982	2 campuses
Dr. Y.S. Parmar University of Horticulure and Forestry	Palampur (H.P.)	1985	1 campus
Indira Gandhi Krishi Vishwa Vidyalaya	Raipur (M.P.)	1987	1 campus
University of Agricultural Science s	Dharwar (Karnataka)	1987	

THE ORGANISATIONS FOR CROP IMPROVEMENT

Organisation And Functions of ICAR

The Indian Council of Agricultural Research (ICAR) is synonymous to agricultural research and education in the country. The council is an autonomous society and has played a crucial role in the development of agricultural reasearch and education. The objectives of the council may be briefly summarised as follows : to promote, guide and coordinate agricultural and veterinary research and education throughout India, to train research workers by offering scholarships, to serve as a clearing house of information in regard to research and toadvise on agricultural and veterinary matters generally, and to undertake the publication of scientific papers, monographs etc.

The Minister and the State Minister of Agriculture and Irrigation are the President and the Vice-President, respectively, of the council. The Director General is the Principal Executive of the council (Fig. 29.1) ; he is also Secretary to the Government of India in the Department of Agricultural Research and Education. He functions as the principal advisor to the government in the matters related to agricultural and veterinary research and education. The council functions through the following bodies.

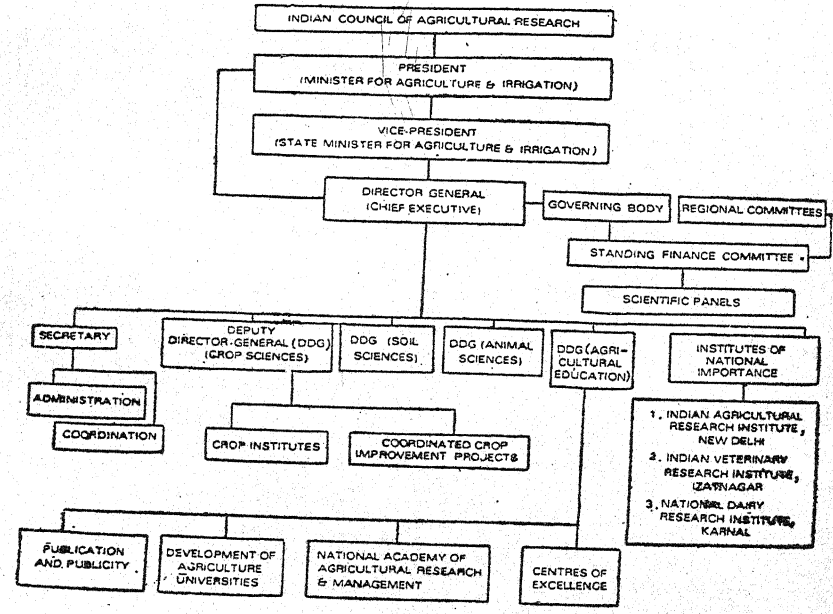


Fig. 29.1. Organisation of ICAR. Details are shown for the organs related to crop improvement

Governing Body. The Director General presides over the Governing Body, which is the chief executive and decision making authority. It consists of agricultural scientists and persons with a knowledge of and interest in agriculture. It makes decisions regarding policies, research projects and schemes, and controls the budget.

Standing Finance Committee. Standing Finance Committee is a subcommittee of the Governing Body and is presided over by the Director General. It examines budget proposals before they are put before the Governing Body.

Norms And Accreditation Committee. The committee consists of 5 Vice-Chancellors of agriculture universities nominated by the President of the Council, and is headed by the Director General. It is responsible for the development of agricultural universities, and maintenance of standards of education in agriculture and animal sciences.

Regional Committees. The conntry is divided into 8 agroclimatic zones ; each zone has a regional committee headed by the Director General. These committees review the status of agricultural research and education in the respective zones and make appropriate recommendations.

Scientific Panels. There are 18 scientific panels for individual disciplines, and 5 interdisciplinary panels. The scientific panels scrutinise research schemes and projects of the disciplines concerned and advise the Governing Body on the technical matters related to research and education.

The council has four Deputy Director Generals for (1) Crop Sciences ; (2) Soils, Agronomy and Agricultural Engineering, (3) Agricultural Education ; and (4) Animal Sciences. The DDGs are assisted by Assistant Director Generals and other Technical Officers. The DDG (Crop Sciences) is responsible for all the projects related to crop improvement.

The council receives a lump sum grant from the Government of India, and the receipts of the agricultural produce cess fund. The council has a separate service cadre, Agricultural Research Service (ARS) for its scientists. The ARS cadre is designed to encourage research activities. Every 5 years, an assessment of the performance of each scientist is made and the scientists are granted either promotion or increments for their achievements.

The crop improvement activities in the country are primarily confined to government and semigovernment institutions ; there are some private organisations engaged in crop improvement activities. All the crop improvement activities (except of private organisations) in the country are directly or indirectly supported, supervised and coordinated by ICAR. There are four main channels by which the council is involved in crop improvement activities : (1) centre institutes on crops, (2) agriculture universities, (3) coordinated crop improvement projects, and (4) *ad hoc* research schemes.

In addition, the council awards a number of prizes in the recognition of outstanding research achievements. The awards are : Rafi Ahmad Kidwai Memorial Prize (1956, 11 awards of Rs. 10,000 each, every 2 years), Dr. P.B. Sarkar Endowment Prize (1971, one award of Rs. 5,000, once in every 3 years), ICAR award for Team Research (1974, 3 awards, every 3 years), Hari Om Ashram Trust Award (1975, 2 awards of Rs. 10,000 each, every year), Dr. R.D. Asana Endowment Prize (1974, one award of Rs. 2,000, every 3 years) and Jawaharlal Nehru Award (1972, 5 awards of Rs. 5,000 each, every year).

The council also provides financial assistance to registered societies in agriculture and animal sciences for the publication of research journals, for conferences, seminars and symposia, for summer institutes and short courses, travelling expenses for attending international conferences etc.

Central Institutes for Crop Improvement

A number of research institutes are engaged in crop improvement (Table 29.4). These institutes are under the control of ICAR. Some of the institutes work on a single crop, *e.g.*, Central Rice Research Institute, while some work on more than one crop, *e.g.*, Central Plantation Crops Research Institute. A brief account of these institutes is given below.

Indian Agricultural Research Institute (IARI). An Agricultural Research Institute was established in Pusa in 1905. Pusa is a village in Bihar, then in the province of Bengal. In 1934, the institute building suffered damage due to earthquake ; the institute was transferred to New Delhi in 1936. In 1947, the name of the institute was changed to the present Indian Agricultural Research Institute. The institute is primarily an educational institution. A two year postgraduate diploma was started in 1923, and in the year 1958 it was granted the status of a deemed university by the University Grants Commission.

The institute has an unparalleled history in the field of agricultural research. Many central institutes owe their origin to research projects initiated at this institute, *e.g.*, SBI, CTRI, CPRI, Indian Lac Research Institute etc. A more recent example is the NBPGR which began as a scheme for plant introduction in the Botany Division of the institute. The institute has 14 stations scattered throughout the country. IARI has developed many high yielding varieties of wheat, jowar, maize, bajra, linseed, arhar, vegetables etc. In addition, basic aspects of crop improvement have been extensively investigated at the institute.

Sugarcane Breeding Institute (SBI). The Sugarcane Breeding Institute was established in 1919, and in 1924 was taken over by the Government of India from the Madras Government. The institute became a part of ICAR in 1962. It has 4 stations at Karnal (Haryana), Cannanore (Kerala), Lucknow (U.P.) and Motihari (Bihar). The

institute is responsible for breeding improved varieties of sugarcane and to conduct basic research on genetics and cytogenetics of sugarcane ; it is also responsible for research on other aspects of sugarcane cultivation. In Coimbatore, sugarcane flowers freely and sets plenty of seed.

The institute has evolved nearly 2,000 varieties of sugarcane ; all Co varieties owe their origin to this institute. These varieties have been extensively used in the country and in some 26 other countries. The sugarcane varieties are produced from complex crosses between *S. officinarum*, *S. spontaneum* and *S. barberi*. Crosses between *S. officinarum* and *S. spontaneum* were initiated by Dr. C.A. Barber (1912-28), the first Head of the Institute ; after him the Indian canes have been named *S. barberi*. The institute has a large germplasm collection with 2,800 entries. A notable achievement of the institute in distant hybridization is a cross between sugarcane and maize, and another between sugarcane and jowar. In 1974, a National Hybridization garden was established in which participating states make desired crosses. Several somaclonal variants from tissue culture have been released as varieties ; this achievement is unique in this country.

Indian Institute of Sugarcane Research (IISR). The institute was established in 1952 and was taken over by ICAR in 1969. In 1970, a Botany and Breeding section was added for sugarcane improvement. The institute has made notable contributions in the methods for sugarcane cultivation. The coordination unit of the All India Coordinated Sugarcane Improvement Project is located at this institute. It also coordinates the All India Coordinated Seed Production Programme for Sugarcane. The institute has 5 stations at Golagokarannath, Captainganj (U.P.), Dimapur (Nagaland), Pravarnagar (Maharashtra) and Rudrur (A.P.).

Cotton Technological Research Laboratory (CTRL). It was established in 1924, and was transferred to ICAR in 1966. The laboratory evaluates the quality of new cotton strains ; in addition, it conducts basic research on quality of cotton. It is recognised by the University of Bombay for postgraduate and/or Ph.D. training in Textile Physics, Biophysics, Textile Technology and Physical Chemistry.

The laboratory has 11 regional units at important cotton breeding centres at Coimbatore, Dharwar, Guntur, Hissar, Indore, Ludhiana, Nanded, Sriganganagar, Surat etc. Preliminary screening of new strains is done at the regional units (over 10,000 samples every year). The laboratory screens over 3,000 samples every year for fibre and microspinning properties and 500 samples are subjected to full spinning tests. The laboratory is the centre for coordinating the technological work under the All India Coordinated Cotton Improvement Project.

Central Institute for Cotton Research (CICR). The institute was established in 1976. The Coimbatore centre of PIRRCOM, established in 1960, was attached to this institute. The institute is designed as a centre for germplasm collection of cotton from which advanced

breeding material would flow to cotton breeders for location-specific selection. The institute has collected so far 4,000 entries of cultivated as well as 28 wild species of *Gossypium*. The Coimbatore centre has evolved many high yielding varieties of cotton. Some outstanding examples are Sujata and Suvin varieties which are comparable to the Egyptian cotton varieties like Giza 45 in their fibre quality. PRS 72 is a short branched, early maturing variety suited for intensive cropping and MCU 5-VT (earlier MCU 5-WT) is resistant to *Verticillium* wilt. Many other varieties have been developed by the Coimbatore centre. The institute also conducts research on other aspects of cotton cultivation.

Jute Agricultural Research Institute (JARI). A Jute Agricultural Research Laboratory was established in Dacca in 1939, which was shifted to Barrackpore during the partition ; JARI was formally established at Barrackpore in 1953. In 1960, the work of JARI was extended to cover mesta, ramie, sisal and sunnhemp, and a programme of seed multiplication was initiated. The Institute has a Ramie Research Station in Assam and a seed multiplication farm at Panagarh, Bud Bud.

The institute is responsible for the improvement of jute, ramie, mesta, sisal and sunnhemp and for other relevant researches on these crops. The institute has evolved a large number of improved varieties of both *Corchorus olitorius* (JRO) and *C. capsularis* (JRC). JRC 212 and JRC 321, evolved during late 1940s are still popular varieties ; JRC 321 is a selection from a local variety Hewti. JRC 321 is early and suitable for lowland conditions. Resistance to premature flowering and to pod shattering has been transferred from Sudan Green, and varieties like Basudev and JRO 524 have been developed. JRO 524 is reported to have the best fibre quality and easy retting. The first ever monoploid in jute was also reported from this institute.

Jute Technological Research Laboratories (JTRL). The laboratory was established in 1938 and was transferred to ICAR in 1965. Among the responsibilities of the laboratory are included : technological research on jute and other long vegetable fibres, and evaluation of fibre quality of new strains. The technological unit of the All India Coordinated Project for Improvement of Jute and Allied Fibres is located at JTRL.

Central Rice Research Institute (CRRI). CRRI was started in 1946, and was transferred to ICAR in 1966. Dr. K. Ramiah, a well known rice breeder, was its first director. The institute has stations at Simligoda (rice and ragi), Kalimpong (W.B.), Nellore (A. P. ; both for disease and pest forecasting) and at Hazaribagh (Bihar, rainfed rice).

The CRRI coordinates collection and evaluation of rice germplasm throughout the country. It also coordinates the rice improvement work in India through the All India Coordinated Rice

Improvement Project (AICRIP). It is recognised by FAO as a world centre for rice germplasm collection ; the Institute has 15,000 entries. Medium-term storage facilities for germplasm collections are being developed. In addition, the institute carries out research on genetics, cytogenetics and all the important aspects of rice cultivation and improvement. The contribution of CRRI is summed up in the observation of Dr. Randhawa that 'rice research in India closely follows the establishment and growth of the Central Rice Research Institute at Cuttack'. The present-day popular varieties like Jaya, Padma, Bala, Vijaya, Sona, Vani, Shakti etc. are some of the notable achievements of CRRI and AICRIP.

Central Tobacco Research Institute (CTRI). The institute was initiated in 1947 ; it was brought under ICAR in 1965. The CTRI has 6 stations: Vadasandur (T.N.) for cigar, cheroot and chewing tobacco; Pusa (Bihar) for hookah and chewing tobacco ; Dinhata (W.B.) for wrapper and hookah tobacco ; Guntur (A.P.) for cigarette tobacco ; Hunsur (Karnataka) for diseases of flue-cured tobacco ; and Kandukur (A.P.). The All India Coordinated Tobacco Improvement Project is located at CTRI. The institute is responsible for tobacco improvement and other related researches on tobacco cultivation. A number of high yielding varieties have been developed by the institute, *e.g.*, Kanakprabha and Dhandayi flue-cured tobacco, and DP 401 chewing tobacco etc.

Central Potato Research Institute (CPRI). CPRI was first established at Patna in 1949 and was transferred to Simla in 1956. The institute has regional stations at Patna, Babugarh (U.P.) Rajgurunagar (near Pune, Maharashtra), Jullundhur (Punjab), Shillong (Meghalaya) and Ootacamund (T.N.) which serve as testing centres for the breeding materials developed ot CPRI. It has wart testing centre at Darjeeling, and four stations for seed production at Fagu (H.P.), Jullundur (Punjab), Daurala and Kodaikanal. The All India Coordinated Potato Improvement Project is located at CPRI.

The institute is devoted to basic and applied research on potato. One of its objectives is to evolve improved varieties of potato for the entire potato growing area of the country, and production and multiplication of disease-free seed. The institute has developed 16 high yielding disease resistant varieties, and has developed the 'seed-plot technique' of seed multiplication for the production of disease-free seed in the plains. It has a large collection of potato germplasm. The CPRI has collaborative programmes with the International Potato Centre, Lima (peru) and with the Scottish Plant Breeding Station, Edinburgh.

Central Tuber Crops Research Institute (CTCRI). The institute was established in 1963 at Sreekaryam near Trivandrum. The objective of the institute is to conduct basic and applied research on tuber crops other than potato, *viz.*, cassava (tapioca), sweet potato, *Dioscorea*, *Amorphophallus*, *Colocasia*, *Coleus* etc. The Institute has a cassava research project financed by the International Development

Research Centre, Canada. The institute is collecting indigenous and exotic germplasm of the root and tuber crops listed above. CTCRI has developed high yielding disease resistant varieties of cassava, *e.g.*, H 97, H 165, H 226, Sree Sahaya and Shri Vishakham ; of sweet potato, *e.g.*, H 41, H 42, H 268, H 633, H 620, OP 1 and OP 2 (OP varieties developed from open-pollinated seed) ; and *Colocasia*, *e.g.*, Kassibugga and Narkatia.

Indian Institute of Horticultural Research (IIHR). The Institute was established in 1967 at the site of the National Hortorium at Hasseraghatta, near Bangalore. It has three stations : Citrus Experiment Station in Gonicoppal, Horticultural Experiment Station in Chethalli (both in Karnataka) and Central Mango Research Station at Rehmankhera near Luckow (U.P.). The responsibility of the institute is basic and applied research on fruit crops, *e.g.*, *Citrus*, mango, grapes etc., and on vegetables. Four promising grape hybrids have been developed which are to be released shortly, *e.g.*, C 36-16, B 11-3, C 2-5 and B 42-23.

Central Soil Salinity Research Institute (CSSRI). The institute was established in 1969 to conduct research on reclamation and management of salt affected (sodic) soils for crop production. The institute has a station at Canning (W.B.). It has three divisions : Soils and Agronomy, Genetics and Plant Physiology, and Engineering. A large number of crop varieties have been screened at the institute and a number of relatively salt tolerant varieties of different crops have been identified. Some of these varieties are listed below.

Rice (early maturing)	Pusa 2-21
Rice (late maturing)	Jaya, IR 8
Wheat	HD 1982 (Janak), WH 157, WL 711, HD 1553, HD 2009
Barley	DL 70, DL 36, B 5105, K 153, K 198, BHS 24
Bajra	PHB 13, HB 3, HB 4

Central Arid Zone Research Institute (CAZRI). The institute was started in 1959 when the Central Research Farm at Jodhpur was reorganised as CAZRI. The Institute is devoted to the development of relevant technologies for a better management of desert and the basic research necessary for the above. The institute is not only multidisciplinary, it also conducts research on animals. The crop improvement activities of the institute are mainly introduction of forest species from foreign countries, evaluation of varieties of various fruit crops and a main centre of the All India Coordinated Millet Improvement Project. Among the introductions, some species of *Eucalyptus* and *Acacia* are suitable for desert afforestation ; *Eucalyptus virdis* is promising for oil production and *Simmondsia chinensis* (introduced from Arizona, U.S.A.) for wax extraction.

Indian Grassland and Fodder Research Institute (IGFRI). The institute was established in 1962 and was transferred to ICAR in 1966. IGFRI aims at developing technology for maximisation of fodder and forage production from various types of soils, including alkaline and saline soils, and under various levels of management, and to transfer this technology to farmers it has a division of plant improvement. The institute also offers training in the production and utilization of fodder.

National Bureau of Plant Genetic Resources (NBPGR). The bureau was established in 1976 when the Plant Introduction Division of IARI was reorganised. The organisation and the function of the bureau have been described in some detail in Chapter 2. Further description will not be given here, except to note that the burea is the sole agency for the introduction and export of germplasm of all plant species.

The Central Plantation Crops Research Institute (CPCRI). The institute was established in 1970 by uniting the Central Coconut Research Stations at Kasaragod and Kanyagulam, and the Central Arecanut Research Station at Vittal. The institute has 5 regional stations, 7 research centres and 2 seed production farms. It is responsible for basic and applied research on coconut, arecanut, cashewnut, cacao, oil-palm, pepper, cardamom, ginger, turmeric and tree spices. It is the headquarter for the All India Coordinated Improvement Project on coconut, arecanut, spices and cashewnut. The institute has developed the T × D and D × T hybrids of coconut that are high yielding and early bearing. In addition, the institute has a long list of achievements to its credit.

Vivekananda Parvatiya Krishi Anusandhan Shala (VPKAS). The Vivekananda Laboratory was started in 1924 in the kitchen room of a house in Calcutta by Prof. Bosi Sen. The laboratory was shifted to Almora (U.P.) in 1936. In 1959, the laboratory was taken over by the Department of Agriculture, U.P., and was finally transferred to ICAR in 1974 under its present name. The initial work was limited to the physiology of living plant cells. But at present the anusandhan shala has sections of physiology, genetics, pathology and chemistry.

ICAR Research Complex for North-Eastern Hills Region (RCNEHR). The research complex was established in 1975 in order to fulfil the research and development needs of the North-Eastern parts of the country, *viz.*, Meghalaya, Tripura, Manipur, Arunachal Pradesh, Nagaland, Mizoram, Sikkim, and the North Cachar and Mikir Hills of Assam. The headquarters of the complex is in Shillong (Meghalaya), and it has 9 stations spread over the areas mentioned above. The responsibility of the complex covers the entire range from agriculture, animal sciences to fisheries.

Central Agricultural Research Institute for Andaman And Nicobar Group of Islands (CARI). The institute was initiated in 1978 with its headquarters at Port Blair. The responsibility of the institute cover

the whole range of agriculture, animal sciences and fisheries. A number of varieties of several crops have been evaluated for their adaptability to local conditions. Feasibility of potato cultivation is also being investigated in some areas.

Agriculture Universities

Agriculture universities are semi-autonomous institutions supported by the respective state governments and the ICAR, but are deemed to be the responsibility of the state governments after one plan period ; they receive financial assistance from ICAR for specific projects. The Agricultural universities have inherited from the Departments of Agriculture of the respective states the responsibility for agricultural education, research and extention ; the research stations and the staff thereof have also been transferred to them. In some states, this change has neither been smooth nor complete. At present, the number of agriculture universities in India is 3 (Table 29.5). It is planned that each state should have one agriculture university ; some states have more than one university, *e.g.*, U.P. and Maharashtra have 4 agriculture universities each.

Agriculture universities are responsible for research and extension activities of the entire state (or part of the state in U.P. and Maharashtra). The research activities in the universities are looked after by (1) teaching staff (primary duty teaching), (2) research staff (primary duty research) at the main campus as well as at the regional stations, (3) staff of ICAR coordinated projects and (5) research personnel in *ad hoc* schemes. In addition, students carry out the major portion of basic investigations. Agriculture universities were established on the concept of integrated teaching, research and development. This integration is sought to be achieved in two ways : each staff member is transferred to teaching, research or extension positions, and each scientist is expected to devote some time to teaching, research and extension with a major emphasis on the primary duty.

Agriculture universities have contributed a great deal to crop improvement activities in the country. A large number of high yielding varieties of a number of crops have been developed by the agriculture universities, *e.g.*, in maize, jowar, bajra, wheat, rice, pulses, fruits and a number of other agricultural and horticultural crops. The examples are too numerous to be listed ; the student has only to look into the extension and research literature put out by his own university to come across a number of notable contributions.

All India Coordinated Crop Improvement Projects

The All India Coordinated Crop Improvement Projects (Table 29.3) provide an efficient channel for multilocation testing of newly evolved strains by the agricultural universities and the central institutes. In addition, the breeders/geneticists employed in the projects

may develop their own materials for inclusion in these tests. The coordinated approach was first used in this country before anywhere else in the world. Each coordinated project is headed by a project coordinator and has a number of centres. The centres are located in agriculture universities and central institutes ; sometimes they may be located in traditional universities, institutions of other scientific organisations, and in private institutions. The locations for centres of coordinated projects are selected on two considerations : the importance of the crop in the region, and the availability of infrastructure for the project. In some cases, the council may develop the infrastructure as alternative locations may not be available. The coordinated projects are financed by ICAR on 100% basis, but in the case of agricultural universities 25% of the expenditure is borne by the state. At present, the coordinated projects are grouped into three categories : project directorates, coordinated projects and coordinated programmes. The nature of activities of all the three categories of projects are the same, only the scope and the magnitude differs.

Project Directorate. A project directorate is headed by a full-time Project Director who is assisted by a number of Associate Project Directors or Associate Project Coordinators and a group of scientists. He also has a coordinating unit with supporting staff. The project directorates perform the functions of the coordinated projects. In addition, they maintain germplasm, organise off-season nurseries, monitor pests and diseases and make forecasts etc.

Coordinated Projects. A coordinated project is headed by a full-time Project Coordinator who is assisted by several Zonal Coordinators and a Principal Investigator for each discipline. The coordinating unit consists of supporting personnel. The functions of the Project Coordinator are to plan, guide, supervise, coordinate and monitor the programmes of the research work under the project.

Coordinated Programmes. Coordinated programmes are smaller than coordinated projects ; they are headed by a Principal Investigator (not a Project Coordinator), and do not have a coordinating unit. They perform the same functions as of the coordinated projects.

The head of a coordinated project (Director/Coordinator/Principal Investigator) is selected on the basis of his scientific achievements. He should be able to command respect from his colleagues and be able to guide and supervise the research activities of the project. The coordinating units of the projects are located in agriculture universities or in central crop institutes.

Functions of Coordinated Projects for Crop Improvement. The coordinated projects serve two basic functions.

1. To evaluate the materials generated by central institutes and agriculture universities under a wide range of agroclimatic conditions and under uniform management.

2. To make recommendations on the suitability of new strains for release as varieties. This is done after a thorough testing for all the important characteristics of the new strains, *e.g.*, yielding ability, disease resistance, quality etc.

In addition, the coordinated projects provide a forum to scientists working on related problems to exchange their views, a means of critical review and replanning the research programmes and an opportunity for the scientists under the project to initiate breeding programmes of their own.

Factors Responsible for The Success of Coordinated Projects. The coordinated projects have been extremely successful. A large number of high yielding, disease resistant varieties have been released through them. The projects are now placing a greater emphasis on minor millets, pulses and oilseeds, and on the improvement of protein content and quality. But the chief objective of the projects remains, and perhaps shall remain in the future, the development of high yielding and disease resistant varieties of various crops. The factors responsible for the success of coordinated projects are briefly summarised below.

1. The country was divided into eight zones on the basis of soil and climate without any reference to political boundaries. This led to a cooperation between scientists working in agriculture universities and central institutes. This has been able to generate considerable enthusiasm among the workers, thus eliminating isolation.
2. Appointment of full-time coordinators of high competence has been responsible for a uniform experimentation of high order.
3. A world collection of germplasm was made available to the breeders at the beginning of the projects. This provided the wide genetic base required for effective crop improvement programmes.
4. The annual (in some cases half-yearly, *e.g.*, rice, pulses) workshops provide an excellent forum for discussion, critical review and repatterning of programmes of the projects. This has enabled the projects to remain flexible and to make necessary changes as needed.
5. A close interaction between scientists working in different disciplines is an integral part of the coordinated projects. This has enabled the breeders to perform their tasks more easily, and the evaluation of new genotypes is more speedy and accurate.

Ad hoc Research Projects

ICAR supports on 100 per cent basis *ad hoc* research projects on various aspects of agricultural and animal sciences. These

schemes are short-term projects, the period not exceeding 5 years in any case. The council provides full recurring expenditure, but not nonrecurring expenditure, except in a few exceptional cases. The council receives funds for *ad hoc* projects from the agricultural produce cess fund (a certain percentage) and from various international agencies, *e.g.*, UNDP, IDRC, SIDA, DANIDA etc.

Proposals for *ad hoc* schemes may be submitted by scientists throughout the year. The scientific panels of the respective disciplines meet twice a year (May-June and November-December) and critically review the proposals. Research schemes approved by the scientific panels are put before the Standing Finance Committee for its consideration. They are finally approved by the Governing Body of the ICAR. *Ad hoc* schemes are sanctioned to agricultural universities, central institutes, traditional universities and private and public institutions.

The *ad hoc* schemes, by their nature, are not designed for crop improvement *per se*. Instead, they are meant for solving specific problems of crop improvement, which is expected to be achieved in a short period of time. Such schemes generally relate to basic aspects of crop improvement, that is, genetic and cytogenetic studies.

SUMMARY

Agricultural research had its beginning in 1871 when a Department of Revenue, Agriculture and Commerce was created by the Government of India. The first scientific staff in the department was appointed in 1982. Between 1901 and 1905, six agricultural colleges were started ; in 1905, an Agricultural Research Institute (now IARI) was established at Pusa, Bihar. ICAI was established in 1929. Before the establishment of ICAR, and upto 1965, a number of Central Commodity Committees looked after research and development of specific crops. These committees had established some of the present-day central institutes. In 1956, ICAR, in cooperation with cotton and oilseeds committees, started PIKRCOM centres which later gave way to the coordinated projects. The first All India Coordinated Research Project was initiated in 1957 for exploitation of hybrid vigour in maize. The success of this project led to the establishment of several other coordinated projects ; at present 26 coordinated crop improvement projects are in operation. The ICAR was reorganised twice (in 1955 and in 1973) leading to its present set up. The first agriculture university was established at Pantnagar (U.P.) in 1960, which stimulated establishment of agriculture universities in other states. At present, there are 23 agriculture universities.

ICAR is the premier body for agricultural research and education in India. The council functions in crop improvement through (1) central crop research institutes (total number 20), (2) agriculture universities (total number 23), (3) coordinated crop improvement projects (total numbers 26) and (4) *ad hoc* research schemes. The central institutes and agriculture universities generate new strains which are evaluated in the coordinated projects. The *ad hoc* schemes are concerned with basic aspects of crop improvement and with solving of specific problems. The coordinated projects are a landmark in agricultural research in the country.

QUESTIONS

1. Compare the following : (*i*) coordinated projects and project directorates, (*ii*) coordinated and *ad hoc* projects, (*iii*) CPRI and CTCRI, (*iv*) IARI and ICAR, and (*v*) agriculture universities and central institutes.

2. Briefly outline the initiation of coordinated projects. List their functions and discuss the factors responsible for their success.
3. Give a brief account of the history of agricultural research and development in India.
4. Briefly outline the establishment and development of ICAR. List its objectives and discuss its role in agricultural research and education in India.
5. Give a list of central institutes for crop improvement. Briefly describe the organisation and functions of any two of the central institutes.
6. Write short notes on the following : (*i*) commodity committees, (*ii*) agriculture universities, (*iii*) PIRRCOM centres, (*iv*) project directorates (*v*) *ad hoc* projects, (*vi*) ICAR, and (*vii*) central institutes for crop improvement.
7. Briefly describe the history of development, functions and contributions of agriculture universities to agricultural research and education in India.

Suggested Further Reading

RANDHAWA, M.S. A History of the Indian Council of Agricultural Research. ICAR, New Delhi.

CHAPTER 30

International Institutes for Crop Improvement

In addition to the national crop improvement programmes, there are ten international institutes and one international agency concerned with improving the agricultural production. Eight of the ten institutes and the international agency are directly or indirectly involved with crop improvement work. These institutes and the agency supplement the national efforts. The international institutes are scattered around the world and are situated in tropical countries. The tropical countries constitute the developing countries or the 'third world'. The agriculture in these countries is not well developed and crop improvement work is generally not highly advanced. They are characterised by poor crop yields due to poor crop varieties and, more particularly, poor crop management. The main objective of these institutes is to increase agricultural production of tropical countries through applied research coupled with extension and educational activities. The functioning of these institutes is supported and supervised by the *Consulatative Group for International Agricultural Research (CGIAR)*.

The CGIAR was established in 1971 by the joint efforts of *Food* and *Agriculture Organisation (FAO)*, the World Bank and the United Nations Development Programme (UNDP). The CGIAR is financially supported by sponsors, governments, development banks, foundations and some other sources. It initiated and established six of the eleven international institutes, and at present it financially supports and supervises all of them. Periodically, CGIAR assesses the progress and the programmes of these institutes with the help of internationally recognised experts in the field of agriculture. The various international institutrs are listed below.

1. International Rice Research Institute (IRRI), Los Banos Philippines.
2. Centro International de Mejoramiento de Maiz y Trigo (CIMMYT), (International Centre for Maize and Wheat Improvement), el Baton, Mexico.
3. Centro International de Agricultura Tropical (CIAT), (International Centre for Tropical Agriculture). Palmira, Colombia.
4. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

5. West African Rice Development Association (WARDA), Monrovia, Liberia.
6. Centro International de Papa (CIP), (International Centre for Potato), Lima, Peru.
7. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), P.O. Patancheru, Hyderabad, India.
8. International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya.
9. International Board for Plant Genetic Resources (IBPGR), Rome, Italy.
10. International Livestock Centre for Africa (ILCA), Addis Ababa; Ethiopia.
11. International Centre for Agricultural Research in Dry Areas (ICARDA), Aleppo, Syria.

International Rice Research Institute (IRRI)

IRRI was initiated in 1960 jointly by Ford and Rockefeller Foundations. This institute is concerned with the improvement of tropical rices. The emphasis of the institute is to develop genotypes suited to tropical Asian countries. Research on other aspects of rice prioduction is also carried out by the institute. In addition, it maintains a huge collection of rice germplasm consisting of more than 42,000 entries. IRRI has a sophisticated cold storage facility for long-term storage of rice germplasm. It has a computerised system of germplasm storage that greatly facilitates the retrieval of desired germplasm. The semidwarf rice varieties were first developed by IRRI from where they were introduced in India.

Centro International de Mejoramiento de Maiz y Trigo (CIMMYT)

CIMMYT was established in 1966 by the joint efforts of Ford and Rockefeller Foundations on the basis of an earlier pragramme. The earlier programme was initiated by Rockefeller Foundation in collaboration with the Ministry of Agriculture of Mexico in 1943. The primary responsibility of CIMMYT is the improvement of maize and wheat. But it is also engaged in the improvement work on other cereals, such as *Triticale*, barley and jowar. In India, contributions of CIMMYT are most apparent in the case of wheat ; Mexican semidwarf wheats are notable examples. CIMMYT has also been involved in the *Triticale* programme of the country. This institute has opted to work on open-pollinated maize varieties instead of hybrid or synthetic maize varieties for obvious reasons. CIMMYT maintains a large germplasm collection of maize consisting of 8,000 entries.

Centro Internacional de Agricultura Tropical (CIAT)

CIAT was established in 1967 by the joint efforts of Ford and Rockefeller Foundations. The primary responsibility of CIAT is

the improvement of cassava and beans. It also looks after maize and rice improvement in collaboration with CIMMYT and IRRI, respectively. The institute also carries out research work on pasture management and farming systems. The research work of the institute is basically directed at solving the problems of Latin America. CIAT has a collection of 10,000 entries in beans, and is fast building up collections of forage grasses and pasture legumes.

International Institute of Tropical Agriculture (IITA)

This institute was established by the collaborative efforts of Ford and Rockefeller Foundations in 1968. The primary concern of IITA is the improvement of grain legumes, and root and tuber crops. It is also engaged in the improvement of maize and rice in collaboration with CIMMYT and IRRI, respectively. Research work on cropping systems is an important aspect of the research activities of the institute. The institute is basically concerned with problems of the African continent; therefore its research activities have an African emphasis. IITA is fast building up germplasm collections of grain legumes, particularly of cowpeas.

West African Rice Development Association (WARDA)

WARDA was established in 1971 by the joint efforts of West African Governments. The Institute looks after rice improvement in collaboration with IRRI and IITA. It also promotes regional cooperative research in rice in the West African countries. The institute is primarily concerned with the development of rice genotypes suited to African conditions.

Centro Internacional de Papa (CIP)

The International Centre for potato was established in 1971 by CGIAR. The institute is responsible for the improvement of potato; particularly for tropical countries. The Institute is also looking after research work on other aspects of potato production. A world collection of potato germplasm is being built up at CIP. There are already 11,000 entries in the germplasm collection ; the number of entries is increasing at a fast rate.

International Crops Research Institute for The Semi-Arid Tropics (ICRISAT)

The ICRISAT was initiated in 1972 by CGIAR to increase the agricultural production in the semiarid tropics. The institute concentrates on the improvement work on jowar, bajra, peanuts, pigeonpea and chickpea. Research work on dryland farming systems is an important aspect of the research activities of the institute. In addition, a programme to exploit the *Rhizobium* legume sybiosis in chickpea, pigeonpea and groundnut is in progress. It is hoped that this would reduce the requirement of nitrogenous fertilizers in the semiarid agriculture. ICRISAT is building up a large collection of cereals and grain legumes.

In 1988, ICRISAT collection had 96,200 accessions, of which over 13,000 were chickpea entries. Several varieties developed by the institute have been released in India, Nepal, Australia, Algeria, Italy etc., *e.g.*, jowar varieties CSV 13 and SAR 1 (in *Striga* endemic areas), bajra variety MP 124, and groundnut variety ICGS 44 etc. in India.

International Laboratory for Research on Animal Diseases (ILRAD)

The institute was established in 1973 by CGIAR. It is concerned with research work on animal diseases.

International Board for Plant Genetic Resources (IBPGR)

IBPGR was established in 1974 by CGIAR to coordinate and promote conservation of plant genetic resources. *It is an agency rather than an Institute.* IBPGR aims to promote the conservation of genetic resources for agriculture and forestry. At present, it supports three regional programmes for near East, Mediterranean and South-east Asia. It also supports several crop-specific local programmes, *e.g.*, rice collection at IRRI, and also provides information on plant genetic resources. IBPGR promotes iniernational cooperation in plant genetic conservation. Fiee exchange of germplasm is a precondition for the support from IBPGR. Genetic conservation efforts require huge funds. In view of limited funds, IBPGR has begun to recognise priority areas for concerted effort. Conservation of tuber and rooot crops is one of the areas of priority.

International Livestock Centre for Africa (ILCA)

ILCA was established by CGIAR in 1974. It concerns with livestock improvement and with livestock production systems for Africa. It also looks after research and development of other animals of economic importance.

International Centre for Agricultural Research in Dry Areas (ICARDA)

ICARDA was established in 1976 by CGIAR. The institute is primarily responsible for the improvement of barley, wheats and lentils. Research work is also done on farming systems. The institute also does research work on the improvement and management of sheep. The research activities of the institute are related to the conditions of the Mediterranean region, particularly dryland conditions.

Functions of International Institutes

The main responsibility of the international institutes is to increase the agricultural production in the tropics. To achieve this, they engage in applied agricultural research and extension activities. These activities of the institutes may be summarised as under.

1. Genetic improvement of the crops concerned to develop high yielding, disease resistant lines better suited to the various environments.
2. Collection and conservation of germplasm of the concerned crops and their relatives.

3. To conduct research on farming systems for an efficient use of the available resources.
4. To determine the appropriate technology suitable for the needs and the resources of the region. The emphasis is to evolve improved practices from the existing ones so that the local farmers are able to adopt the new improved technology with as little difficulty and expenditure as possible.
5. Extension activities to popularise the new technology so that the cultivator is able to adopt them.

Of these activities, the genetic improvement of crops and the germplasm conservation are of our immediate interest. Other activities, though important, are beyond the scope of this book. Many of these institutes, *e.g.*, IRRI, ICRISAT, CIMMYT etc., have the responsibility for breeding crop varieties for several tropical countries. The soil, climate, agricultural practices, prevalent diseases and consumer preferences would vary to a great deal from one country to another, and often within a single country. Development of varieties suited to these varied conditions poes a problem which the breeders have never faced before. The breeders have attempted to solve this problem in the following manner.

1. The first step consists of the identification of the possible environments for which the varieties have to be developed. In each environment a location is selected for the evaluation of breeding materials and varieties.
2. The second step involves making of a large number of crosses between parents with very wide genetic base. This is done at a very large scale. It is hoped that segregating materials from promising crosses would perform well in one or the other environment.
3. Finally, the segregating materials from the promising crosses are tested in the various environments at different locations. A number of lines suited to those particular conditions are identified and selected.

Emphasis is laid on testing the breeding material under poor and good management conditions so that genotypes suitable for these conditions are identified. The promising lines are freely available to all the countries for direct release as varieties or for use in local programmes on crop improvement. The international institutes no more evolve varieties and promote them. This is in response to the criticisms levelled against them in connection with the promotion of semidwarf wheat and rice varieties by IRRI and CIMMYT.

The role of international institutes in genetic conservation cannot be overemphasised. Genetic conservation has been the responsibility of local organisations. Due to a lack of coordination, there has been an inevitable duplication of efforts resulting in a wastage of time, energy and funds. This has also limited the extent to which

variability in crop species could be collected and conserved. In addition, there were bound to be restrictions on germplasm exchange due to political and other reasons. The international institutes coordinate their germplasm collection activities with various similar national organisations. This permits a greater efficiency in genetic conservation efforts. IRRI has established a computerised germplasm bank for rice which makes it much easier to locate and obtain the desired lines from the bank. Free exchange of genetic materials ensures their maximum utilization in crop improvement projects.

Some Contributions of Intarnational Institutes

The international institutes have contributed to a great extent in the agricultural development of the third world countries. The increased agricultural production in India is largely due to the development of semidwarf wheat and rice varieties. These varieties originated at CIMMYT and IRRI from where they were introduced in India. Lerma Rojo and Sonora 64 wheats were direct introductions from CIMMYT. Kalyan Sona and Sonalika were selected from segregating generation materials introduced from the same institute. Smilarly, IR 8, IR 24, IR 28 and IR 36 varieties of rice were introduced from IRRI, Philippines. Many of semidwarf rice varieties developed in this country have IR 8 as one of their parents. Although the contributions of the international institutes are more known in the case of rice and wheat improvement, their contributions in the improvement of other crops are also considerable.

It may be relevant to briefly examine the validity of the so called *green revolution* associated with the development of semidwarf wheat and rice varieties. These semidwarf varieties rapidly occupied very large areas (approximately 10 million hectares in each case) in the tropical countries, particularly in India, Pakistan and Philippines. There was a considerable increase in the production of these crops in the countries concerned. This increase raised false hopes of increase in prosperity and continuous rise in agricultural production. Introduction of these varieties led to a number of socioeconomic problems in these countries which are summarised below.

1. The quality of these varieties was not liked by people. For example, the red colour of the Mexican wheats and the cooking quality of IR 8 rice were not liked by people in India. But these defects have since been acceptably removed by breeding work within this country and a number of semidwarf varieties are now very popular with the cultivators.
2. These varieties were susceptible to certain diseases which often caused considerable loss to the cultivators. Susceptibility of IR 8 to bacterial leaf blight is a case in point, The poor farmer was often the greatest sufferer because he could not afford timely plant protection measures. The rich farmer, on the other hand, generally did not suffer to that extent becaus-

he could adopt costly plant protection measures to protect the crop against diseases and pests.

3. Single pureline varieties came to occupy large areas which increased the chances of epidemic development. Luckily, there has been no serious epidemic so far, although local losses did occur due to diseases. But this situation was very risky and has now been corrected to some extent.

4. The most serious effect of the green revolution was the increase in the gap between the rich and the poor farmers. Increase in income led to more mechanisation and an increase in unemployment. The rich farmers became richer and bought the land from poor farmers. This caused a considerable increase in the number of landless labourers.

Thus the green revolution appears to have failed in the sense that it could not fulfil the rosy promises that it once held out. But it has succeeded to the extent that it increased the agricultural production in several countries making them almost self-sufficient in foodgrain production. In addition, it has brought into sharp focus the inadequacies of a hasty "green revolution" in the third world. This has emphasised a more cautious and well planned programme for agricultural development that would create the minimum of socioeconomic problems.

SUMMARY

There are 11 international organisations for agricultural research. Nine of these organisations are engaged in crop improvement activities. Some of them are highly specialised and look after only one crop, *e.g.*, IRRI for rice and CIP for potato. But most of them are involved with many crops. The chief objective of these institutions is to increase agricultural production in tropical countries. Their activities include crop improvement, germplasm conservation, to conduct research on farming systems, to evolve technology suited for the needs and the resources of the region and extension education to assist the farmers to adopt the new technology. These institutes have contributed considerably to the increase in the agricultural production in the tropical countries. This contribution is particularly clear in the case of wheat and rice. The new dwarf varieties of these two crops were responsible for the so called 'green revolution' that made India self-sufficient in foodgrains. But green revolution was associated with many agricultural and socioeconomic problems. This experience has made it abundantly clear that the technology to be used by the farmers in these countries should be suitable for their needs and resources, *i.e.*, it should be developed from the technology existing in the region and not imported from a totally different situation.

QUESTIONS

1. List the various international agencies and institutes concerned with the improvement of agricultural production. Briefly describe the objectives and the functions of any two of them.

2. Discuse the functions of international institutes. Discuss the chief contributions made by them in solving the problem of low agricultural productivity in tropics and subtropics.
3. Write the full names of the following : CGIAR, CIAT, CIMMYT, CIP, FAO, IBPGR, ICARDA, ICRISAT, IITA, ILCA, ILRAD, IRRI, UNDP, WARDA.

Suggested Further Reading

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Glossary

Adaptation. It is the process by which organisms become more suited to survive and function in a given environment. It also refers to the result of this process.

Addition Line. An addition line has one pair of chromosomes from another variety or species in addition to the normal somatic chromosome complement of the species.

Agronomic Trials. Evaluation of new strains for release as a variety ; an entry showing superior performance in the first year of URT is included in trials designed to determine the optimum date of sowing and irrigation level ; under the respective All India Coordinated Crop Improvement Projects.

Alien-Addition Line. It has one pair of chromosomes from a related wild species in addition to the normal somatic chromosome complement ($2n$) of the species.

Allele. Alleles are alternative forms of the same gene, and are located at the same point (locus) in homologous chromosomes.

Allogamy. In allogamy, pollen grains from flowers of one plant pollinate the flowers of other plants (*Syn.*, cross-pollination).

Allopolyploid. A polyploid containing two or more different genomes.

Amphidiploid. It has two copies of each of the two or more different genomes present. Thus an amphidiploid has the somatic chromosome complement of two or more diploid species.

Aneuploid. An aneuploid organism has a chromosome number that is not an exact multiple of the basic chromosome number (x).

Anther Culture. Culture of anthers (or pollen grains) on a suitable medium for the production of callus and/or haploid plants.

Anthesis. The first opening of a flower.

Antibiosis. The adverse effect of feeding on a resistant host plant on the development and reproduction of the inseet pest.

Apogamy. Development of embryo from synergids or antipodal cells without fertilization ; a form of apomixis.

Apomixis. Development of embryo (and seed) without fertilization.

Apospory. A form of apomixis in which the embryo sac develops from a vegetative cell of the ovule.

Artificial Selection. Selection by man.

Asexual Reproduction. Asexual reproduction does not involve the fusion of male and female gametes.

Autogamy. Pollen grains of a flower pollinate the same flower (*Syn.* self-pollination).

Autopolyploid. A polyploid that has more than two copies of the same genome.

Avoidance. Escaping of a susceptible host plant from damage due to an inseet pest, usually, as a result of the host plants being at a much less susceptible developmental stage when the pest population is at its peak.

B_1 and B_2. Backcrosses of F_1 to P_1 (first parent) and P_2 (second parent), respectively, of the hybrid.

Backcross. A cross between a hybrid and one of its parents. Also to make such a cross.

Backcross Breeding. A breeding method based on repeated backcrossing of the F_1 and the subsequent generations to the recurrent parent.

BC_1, BC_2, BC_3 etc. Progeny from $F_1 \times$ recurrent parent, $BC_1 \times$ recurrent parent, $BC_2 \times$ recurrent parent etc., respectively.

Basic Number. The *haploid* chromosome number of the ancestral diploid species of a polyploid. Represented by x. Also haploid chromosome number of diploid species. x chromosomes constitute a genome.

Basic Seed. Seed produced by mass selection (with progeny test) in a pureline variety or clone ; source of breeder seed.

Biometry. It consists of the application of statistical procedures to biology.

Biotype. Strains of a species of pathogen, particularly an insect pest, differing in their ability to attack different varieties of the same host species (*Syn.* physiological races).

Biparental Cross Analysis. A design for genetic analysis based on progenies derived from the matings involving a random sample of F_2 or later generation plants from a cross between two purelines ; the four designs based on such progenies are NCDI, NCDII, NCDIII and TTC.

Bivalent. Structure formed by pairing of two homologous chromosomes during meiosis.

Breeder Seed. Seed produced by the breeder or the institute which developed the variety. Source of foundation seed.

Bridging Species. A species used in gene transfer from one species to another sexually incompatible species ; bridging species is compatible with both the donor and recipient species ; it may be a natural or synthetic species.

Bud Mutation. Mutation in somatic tissues, usually affecting an axillary bud.

Bud Selection. A form of clonal selection in which mutant buds are selected.

Bulk Method. In this method, F_2 and the subsequent generations are grown in bulk, usually without artificial selection. In the end, pureline varieties are developed through individual plant selection.

Certified Seed. Seed produced from foundation, registered or certified seed. Its purity is certified by a seed certification agency, and is usually used for commercial crop production.

Centres of Diversity. Areas where cultivated plant species and/or their wild relatives show much greater variation than anywhere in the rest of the world.

Centres of Origin. Areas where cultivated plant species are supposed to have originated. Based on centres of diversity.

Centromere. The localised region of a chromosome where spindle fibres attach, and which is responsible for chromosome movement during cell division.

Character. A morphological, anatomical or physiological feature of an organism ; usually a product of the actions of both genotype and environment.

Chiasma. The point of contact between nonsister chromatids of homologous chromosomes during and after diplotene (meiosis).

Chimera. An individual that is composed of cells of two or more genotypes.

Chromatid. Substructures of chromosomes produced by replication of preexisting chromatids. Each chromosome has two chromatids which appear fused at the centromere.

Chromosome. Thread like structures present in nucleus which show distinct change in their morphology during cell division. They are deeply stained with some dyes, particularly with the Feulgen's reagent. Genes are located in chromosomes in a linear order.

Chromosome Manipulation Techniques. Techniques used for promoting gene transfers from chromosomes of a related species into those of a cultivated species.

Chromosome Substitution. The procedure or the process of replacing one pair of chromosomes of a variety with those of another variety or a related species. The latter is *alien substitution.*

Cleistogamy. Flowers do not open at all, *i.e.*, there is no anthesis.

Clone. Individuals obtained from a single plant through asexual reproduction (produced by mitotic cell division).

Clonal Multiplication. Multiplcation through asexual reproduction.

Clonal Selection. A method of selection based on clones. It does not involve sexual reproduction.

Coefficient of Variation. The ratio of standard deviation of a sample to the mean of that sample ; usually expressed in per cent. $[(s/\bar{x}) \times 100]$.

Combination Breeding. It involves the transfer of one or a few characters, usually governed by oligogenes, from one variety to another.

Combining Ability. Ability of a strain to produce superior progeny upon hybridization with other strains.

Competitive Ability. The ability of a genotype to survive in a mixture of several genotypes.

Complex Cross. See *Convergent Cross.*

Composite Cross. A hybrid population generated from a large number of purelines either by compositing equal seeds from the F_1 or F_2 generations from all possible crosses among them, by ordering them into a complex cross, or allowing them to cross with a male sterile line and harvesting the seeds from only the male sterile plants in each generation.

Composite Varieties. Varieties produced by open-pollination among a number of outstanding strains usually not tested for combining ability with each other.

Contiguous Control. A control or check variety planted in an adjacent plot ; a design for the evaluation of a large number of lines/progenies in which the control variety is planted in every 5th or 7th row/plot (*augmented design*), and the comparisons of the line/progeny means are made with the control.

Control. In plant breeding ; a known variety used for comparing the performance of newly developed strains during their evaluation.

Convergent Cross. A cross involving more than two parents (*Syn.*, complex cross).

Coordinated Varietal Trial (CVT). Multilocation trials (usually for two years) conducted under the respective All India Coordinated Crop Improvement Projects for the evaluation of performance of newly developed strains ; in many crops, one year of IET precedes CVT. (Syn., URT, Uniform Regional Trial).

Correlated Response to Selection. Change in one or more quantitative characters due to a selection for another character.

Coupling Phase. Linkage between dominant alleles of two or more genes.

Cross. Mating of two or more individuals or strains having different genotypes. Also the product of such a mating.

Cross-Pollination. See *allogamy.*

Crossing Over. Exchange of homologous segments between non-sister chromatids of homologous chromosomes ; takes place during pachytene.

Cytogenetics. Study of chromosomes in relation to genetics.

Cytoplasmic Inheritance. Transmission of characters through cytoplasm. It is due to the DNA present in cytoplasmic organelles.

Cytoplasmic Male Sterility. Male sterility showing cytoplasmic inheritance.

Cytoplasmic-Genetic Male Sterility. Cytoplasmic male sterility for which a restorer gene is available/known.

Deficiency. Loss of a part of a chromosome.

Detasselling. Removal of the tassel (the male inflorescence) before it sheds pollen ; in maize. An easy method of emasculation.

Diallel Cross. Crossing of a number of genotypes in all possible combinations.

Diallel Selective Mating Scheme. A breeding scheme in which several selected purelines are mated according to the diallel scheme ; the promising F_2 populations are advanced by selfing for the isolation of purelines to be used as varieties ; simultaneously, the selected plants in the F_2 generations are intermated to generate populations which will again be handled as above. This process continues.

Dichogamy. Maturition of male and female reproductive organs of a hermaphrodite flower at different times.

Dihaploid. Haploid derived from tetraploid, *i.e.*, monoploid for two distinct genomes.

Dihybrid. An individual heterozygous for two genes.

Dioecious. Plant species in which unisexual (male and female) flowers occur on different plants. Such species have male and female plants.

Diploid. An organism having two genomes, *i.e.*, with chromosome number of $2x$.

Diplospory. A form of apomixis in which the embryo sac develops from the megaspore.

Diplotene. Stage of meiosis following pachytene in which homologous chromosomes forming a bivalent (or multivalent) begin to move away from each other. Chiasma is seen during this stage.

Disease. An abnormal condition produced by an organism.

Disjunction. Separation of chromosomes at anaphase.

Distant Cross. See *Wide Cross.*

Distant Hybridization. Hybridization between individuals belonging to two different species of the same genus or of different genera.

Disomic. An individual with two homologues for each chromosome of the genome or genomes.

Dockage. Per cent impurity in a seed sample.

Dominance. Ability of an allele to express itself in the heterozygous state.

Domestication. The process of bringing a wild species under human management.

Donor Parent. In backcross breeding ; the parent from which one or few genes are transferred to the recurrent parent (*Syn.* non-recurrent parent).

Double Cross. Cross between two single crosses (*i.e.*, between two F_1's from two single crosses).

Doubled Haploid. A plant or line obtained by doubling the chromosome number of a haploid plant/individual.

Doubled Haploid Technique. A technique in which homozygous lines are obtained by doubling the chromosome numbers of haploid plants extracted from heterozygous (usually F_1) plants.

Drift. Random change in gene and genotype frequencies in small random mating populations.

Duplication. Occurrence of a chromosome segment more than twice in a diploid chromosome complement. The duplicated segment may occur in the same or in a different chromosome.

Early Testing. In self-pollinated crops, evaluation of the performance of early segregating generations (usually, F_2 or F_3 bulks) or F_3/F_4 individual plant progenies in order to assess the worth of purelines that would be isolated from them ; in cross-pollinated crops, the estimation of GCA or SCA of plants/progenies early in the the processs of inbreeding.

Emasculation. Removal of immature anthers (or androecium) from a hermaphrodite flower.

Embryo Culture. Removal of developing embryo from seed and its cultivation in vitro.

Embryo Sac. Cell derived from megaspore. It contains egg cell, synergids, antipodals and polar nuclei (*Syn.*, megagametophyte).

Entry. A newly developed line/strain included for evaluation in the multilocation trials of the various All India Coordinated Crop Improvement Projects.

Environment. Sum total of external conditions which influence an organism.

Epidemic. Development of a disease from a low infection level to a high intensity of infection. *Artificial epidemics* are created by man by providing the inoculum and the suitable environment for the disease development and used in disease resistance tests.

Epiphytotic. See *Epidemic.*

Epistasis. Interaction between two different genes, that is, expression of one gene is affected by another gene and *vice-versa.*

Error Variance. Variance due to factors beyond the control of experimenter. (It is used as the denominator in F-test).

Escape. More commonly *disease escape.* Phenomenon of susceptible plants avoiding disease attack. Also plants showing escape.

Euploid. Individual whose chromosome number is an exact multiple of the basic number.

Evaluation. The process of assessing the performance (yield, quality, disease and insect resistance, etc.) of newly developed strains of a crop through appropriate multilocation trials.

Excitation. Movement of an electron to an outer orbit of a higher energy level.

Exotic. A variety or species introduced from a foreign country.

Exploration. A trip for collection of germplasm of cultivated and related wild species (*Syn.* Expedition).

Expressivity. Ability of a gene to express itself uniformly in all the individuals that carry it in the appropriate genotype.

Extreme Homozygote. A homozygote isolated from a hybrid, usually produced by crossing two or more parents, that has either all the positive or negative alleles for a trait for which the hybrid was heterozygous.

F_1. The progeny obtained by crossing two different genotypes. The first generation from a cross.

F_2. Progeny obtained by self-fertilization of or intermating among F_1 individuals.

F_3, F_4 *etc.* Selfed progeny of F_2, F_3 etc., respectively.

Factor. See *Gene*.

Family. A group of individuals sharing a common parent/ancestor.

Fertility. Ability to produce viable progeny.

Fertilization. Union of male and female gametes in sexual reproduction.

Foundation Seed. Seed produced from breeder seed. It is the source of registered and certified seeds.

Free Radical. Electrically neutral molecules with an unpaired electron in the outer orbit. Free radicals are highly reactive.

Gamete. A specialised cell produced by gametogenesis that participates in fertilization. Gametes are produced through meiosis.

Gametic Chromosome Number. Chromosome number present in gametes of a species.

Gametogenesis. Production of gametes from spores.

Gene. *Functionally*, gene is unit of inheritance ; one or more genes control the expression of a character, and one gene codes for a polypeptide. *Structurally*, gene is a segment of DNA which codes for one polypeptide, ribosomal or transfer RNA.

Gene Bank. Large collection of germplasm representing materials from various parts of the world (*Syn*., World Collection).

Gene Frequency. The proportion of an allele present in a population, represented by p and q.

Gene Interaction. Modification of expression of a gene by one or more nonallelic oligogenes.

Gene Pool. Sum total of genes present in a Mendelian/panmictic (random mating) population.

Gene Sanctuary. An area within the centre of diversity protected from human interference.

Genetic Advance (*under selection*). Improvement in the performance of selected lines over the original population.

Genetic Emasculation. Use of genetic factors to make the male gamete nonfunctional in self and or cross-pollination.

Genetic Equilibrium. In a random mating population ; the stage in which genotype frequencies do not change from one generation to another. It has reference to one or more genes.

Genetic Erosion. Gradual disappearance of various forms of a cultivated species and of its wild relatives.

Genetic Load. The sum total of deleterious (harmful) alleles present in a random mating or Mendelian population.

Genetic Purity of Seed. Freedom of seed from seeds of weeds, other crops and other varieties of the same crop.

Genetic Variance. Variance due to genotypes of different plants or strains. It has additive, dominance and epistatic components.

Genetic Vulnerability. The susceptibility of most of the cultivated varieties of a crop species to a disease, insect pest, or some other stress due to similarities in their genotypes ; usually, due to the presence of one (generally more) common parent(s) in their ancestry.

Genetics. A study of the mechanism of transmission of characters from parents to offspring, origin of variation and gene action.

General Combining Ability. Average performance of a strain in a series of cross combinations. Estimated from the performance of 'F_1's from the crosses.

Genevation Mean Analysis. The estimation of additive, dominance and epistatic gene effects from the means of certain specific families, usually, P_1, P_2, F_1, F_2, B_1 and B_2 ; F_3 may be used in the place of B_1 and B_2, but the *j* effect cannot be estimated in such a case.

Genome. Genome consists of all the chromosomes of a diploid species that are distinct from each other with respect to their gene content and, often, morphology. Members of a genome do not pair.

Genotype. Genetic constitution of an organism.

Germplasm. The sum total of hereditary material or genes present in a species.

Germplasm Complex. Open-pollinated progeny from a mixture of a number of strains of diverse origin.

Haploid. An individual with the gametic chromosome number.

Heritability. The proportion of total phenotypic variance that is due to genotype.

Hermaphrodite. A flower having both male and female reproductive organs.

Heterogenous. A mixture of different types, usually different genotypes.

Heterocaryosis. In fungi, a condition in which a hypha has nuclei of two different genotypes.

Heteroploid. An individual with a chromosome number other than the normal diploid ($2x$) number.

Heterosis. Superiority of F_1 over the parents (or even inferiority to both the parents, *e.g.*, earliness). (*Syn.*, Hybrid Vigour).

Heterostyly. Occurrence of styles and stamens of different lengths in flowers from different plants of a single species.

Heterozygous. An individual having dissimilar alleles of a gene.

Homoeologous Chromosomes. Partially homologous chromosomes generally show reduced or lack of pairing.

Homologous Chromosomes. Chromosomes identical with each other in gene content and morphology ; two homologous chromosomes pair to form one bivalent during meiosis.

Homogeneous. Consisting of individuals of the same genotype or phenotype.

Homozygous. An individual having two or more identical alleles of the same gene.

Horizontal Resistance. Resistance governed by polygenes and is pathotype nonspecific.

Host. The organism attacked by a pathogen.

Hybrid. Progeny from hybridization between two or more strains.

Hybrid Substance. New isozyme present in the hybrid which is different from those present in the parents.

Hybrid Varieties. A hybrid variety is F_1 generation from a cross between two different strains.

Hybrid Vigour. See *Heterosis.*

Hybridization. Mating of two different strains.

I_1, I_2, I_3 *etc.* Generations after one, two, three etc., respectively, generations of inbreeding.

Identification of A Variety. Recommendation by the annual workshop of the concerned All India Crop Improvement Project for the release of a strain or an entry as a new variety.

Immune Reaction. Complete absence of symptoms of a disease.

Improved Seed. Seed of an improved variety having a very high genetic and physical purity, and germination.

In vitro Techniques. The technique of culturing plant cells and organs on artificial media in vitro.

Inbred Line. A nearly homozygous line developed by continued inbreeding, usually selfing, accompanied by selection.

Inbreeding. Mating of individuals more closely related by ancestry than would be expected under random mating.

Inbreeding Depression. Loss in vigour due to inbreeding.

Inbred-Variety Cross. Cross between an inbred and an open-pollinated variety.

Infestation. The attack by an insect pest on the host plant.

Initial Evaluation Trial. The preliminary evaluation of a relatively large number of newly developed strains in the multilocation trials of the concerned All India Coordinated Crop Improvement Project ; the purpose is to reduce the number of strains to a manageable level for a relatively more extensive evaluation in URT or CVT ; ommitted in crops where the number of entries is not large.

Insect pest. An insect species which causes damage to a crop species ; they may be tissue feeders or sucking pests.

Insect Resistance. The property of a host variety to be attacked by an insect pest to a noticeably smaller degree than some other varieties of the same host species.

Intergenotypic Competition. Competition between or among plants of different genotypes ; important in the segregating generations, varietal mixtures, etc.

Interplant Competition. Competition among plants of the same genotype ; important in the stands of purelines, single cross hybrids, clones etc.

Inert Matter. In a seed sample ; all nonliving matter, disease and insect infested seeds and broken (damaged) seeds.

Interspecific Hybridization. Hybridization between plants belonging to two species of the same genus.

Introduction, Plant. Taking a variety or a species into an area where it was not grown before ; even within a country.

Introgression. Transfer of a few genes from one species into the full diploid chromosome complement of another species.

Inversion. A segment of a chromosome is rotated by 180° so that the gene order on that segment is reversed.

Ion An atom that has lost or gained an electron in comparison to the number of electrons normally present.

Ionization. Loss or grain of an electron by an atom.

Irradiation. Exposure of biological materials to radiations, more particularly to mutagenic radiations.

Isogenic Lines. Lines identical in genotype except for one gene.

Isolation. Separation of two or more plants, strains or populations to prevent mating among them. Usually achieved by distance and/or border rows.

Kinetochore. See *Centromere.*

LD_{50}. The dose of a mutagen (or any other agent) which will kill 50 per cent of the treated individuals.

Lethal Gene. Usually recessive ; lethal genes kill each and every individual that carries them in the appropriate genotype.

Line. A group of individuals having common parents or ancestors.

Line × Tester Analysis. The estimation of GCA and SCA effects and vnriances from progenies developed by mating each of the several lines to each of the same selected testers ; additive and dominance variances are estimated from GCA and SCA variances.

Linkage. Tendency of two or more genes to be inherited together.

Linkage Map. A linear (or circular) map of chromosomes showing the position of various genes present in them as determined by their linkage relationship.

Locus. The position at which a gene is located in a chromosome.

M_1, M_2 M_3 *etc.* After treatment with a mutagen ; first, second, third etc. generations, respectively, derived by selfing or asexual reproduction.

Maintainer Line. Line used for maintaining a cytoplasmic male sterile line. It has the same nuclear genotype as the male sterile line.

Male Sterility. Absence of functional male gametes (pollen grains in plants).

Mass-Pedigree Method. In this method, the population is maintained as a bulk till such time when conditions suitable for selection occur ; at this stage pedigree method of breeding is applied.

Mass Selection. In mass selection, several plants are selected on the basis of their phenotype and their seed is composited to raise the next generation.

Mating System. Scheme according to which individuals or lines are mated to produce sexual progeny.

Mean. It is the arithmetic average of a set of observations. $[(\Sigma X)/N]$

Megagametogenesis. Production of the female gametophyte (embryo sac) from megaspore (through mitosis).

Megasporogenesis. Production of the female spore (megaspore) from megaspore mother cell (through meiosis).

Meiosis. Two cell divisions, occurring one after another, after only one DNA replication ; shows chromosome pairing and chromosome separation in the first division and chromatid separation in the second. Leads to the production of haploid cells (spores/gametes).

Meristem Culture. Cultivation of apical meristems, particularly shoot apical meristem, for the production of shoots and plantlets.

Mericloning. Vegetative multiplication through meristem culture.

Metaphase. A stage during cell division during which the chromosomes lie at the metaphase plate (equatorial plate).

Metaxenia. Effect of pollen grains on maternal tissues of fruits.

Microcentre. Areas within centres of origin that show greater diversity than the remaining centre of origin.

Microgametogenesis. Production of the male gametophyte from microspore (through mitosis).

Microsporogenesis. Production of pollen grains (microspores) from microspore mother cell (through meiosis).

Migration. Movement of individuals from one population into another.

Mitosis. Cell division in which the two chromatids of each chromosome move to the opposite poles producing two $2n$ daughter nuclei. Every mitotic division follows DNA replication (compare with meiosis).

Modifying Genes. Genes with no phenotypic effect of their own, but change the expression of some oligogene. They have small, cumulative effect producing a continuous range of phenotypes.

Monoecious. A plant having both male and female flowers.

Monohybrid. An individual heterozygous for one gene.

Monoploid. An individual with the basic chromosome number (x), *i.e.*, with one genome.

Monosomic. An individual with one chromosome less than the somatic chromosome number ($2n-1$).

Multiline Varieties. Mixtures of several similiar purelines having different genes for disease resistance.

Multilocation Trials. Trials conducted at several locations under the various All India coordinated crop Improvement Projects.

Multiple Alleles. More than two alternative forms of a single gene.

Multivalent. The structure formed by the association of more than two homologous chromosomes as a consequence of synapsis during meiosis.

Mutagen. A chemical or physical agent that induces mutation.

Mutagenesis. Induction of mutations with the aid of mutagens.

Mutations. A sudden heritable change in a characteristic of an organism. *Macromutation* produces a sufficiently large change to be easily identifiable, while *micromutations* produce small changes (usually in quantitative traits) and are studied in terms of mean and variance.

Mutation Breeding. Breeding method utilizing variation created by mutagenesis.

n. Gametic chromosome number of a species.

2n. Somatic chromosome number. The chromosome number present in somatic cells of a species.

National Evaluation Trial (NET). In oilseeds ; the plot size is three times the size of that in CVT ; the entries are identified for prerelease multiplication only after NET.

National Trial. In some crops, *e.g.*, wheat ; the topmost entry in CVT or URT of each zone is evaluated in an IET throughout the country to assess if an entry developed in one zone may perform well enough for release as a variety in another zone of the country.

Natural Selection. Selection due to natural forces, *i.e.*, environment.

Noblisation of Sugarcane. Introgession of genes and chromosomes from noble canes and wild *Saccharum* species into Indian canes (*S. barberi*).

Nonpreference. A host variety being unsuitable or unattractive to an insect pest for colonization, oviposition or both.

Nonrecurrent Parent. In backcross method ; the parent from which one or few genes are transferred to the recurrent parent (*Syn.*, donor parent).

North Carolina Design I (NCDI). A mating design used for genetic analysis ; four random plants are mated as females to one random plant used as male to generate one male *group* ; four male groups make a *set* and several sets may be generated in one experiment ; applicable to the segregating generations (self-pollinated species) or random mating population.

North Carolina Design II (NCDII). Equal number of male and female plants selected randomly and mated in a diallel fashion to yield $m \times f$ number of crosses (m=number of males, f=number of females); applicable to the segregating generations (self-pollinated species) or random mating populations.

North Carolina Design III (NCD III). Several random plants selected from an F_2 are backcrossed to the two parents of the concerned F_2 generation.

Notification of a Variety. A notification from the Director, High Yielding Varieties, Ministry of Agriculture and Irrigation, Government of India, to the concerned authorities for the seed multiplication of a variety just released for cultivation by the central or a state Variety Release committee.

Nucleus Seed. See *Basic Seed.*

Nullisomic. An individual having a pair of homologous chromosomes less than the somatic chromosome number of the species ($2n-2$).

Off-season Nursery. Growing the breeding materials during the off-season (a season in which the crop is not grown in the concerned locality) in a location suitable for the purpose; generally to advance the generation.

Oligogenes. Genes having large individual effects producing distinct phenotypes.

Open-Pollination. In cross-pollinated species, pollination occurring naturally without restriction.

Outcross. Natural cross between two different genotypes.

P_1, P_2, P_3 etc. First, second, third etc. parents, respectively, used for producing a hybrid or a series of hybrids. Also the first, second, third etc. generations of parents obtained by selfing.

Pachytene. Stage in meiosis; when pairing between homologous chromosomes is complete giving a single-stranded appearance to the bivalent. Crossing over occurs during this stage.

Panmixis. Random mating without restriction.

Parameter. A numerical quantity which describes some characteristic of a population.

Path Analysis. A standardised partial regression coefficient analysis which splits the various correlation coefficients into the measures of direct and indirect effects of a set of independent variables on a dependent variable (usually, yield).

Parthenogenesis. Egg cell of an embryo sac derived from megaspore develops into an embryo without fertilization.

Pathogen. An organism producing a disease.

Pathotype. Strain of a pathogen virulent toward a specific resistance gene of the host.

Partial Diallel Analysis. A diallel analysis based on a specified sample of crosses from among the all possible crosses (diallel scheme) among a number of parental lines.

Pedigree. A record of ancestry.

Pedigree Method. A method of breeding in which individual plants are selected in F_2 and subsequent generations and a pedigree record is maintained. The pedigree record may be used as a basis for selection in later generations.

Penetrance. Ability of a gene to express itself in the individuals carrying that gene in the appropriate genotype. Penetrance may be complete or incomplete.

Phenotype. External appearance of an individual with reference to a single character or a number of characters.

Physical Purity. Freedom of seed from inert matter, including broken seeds.

Physiological Races. Strains of a pathogen species that differ in their ability to attack different varieties of the host species. Unlike pathotype, it has no reference to the resistance genes.

Plant Breeding. The branch of biology concerned with changing the genotype of plants so that they become more useful to man.

Pleiotropy. Phenomenon of a single gene affecting two or more different characters.

Pollen Culture. Cultivation of pollen grains in vitro for producing haploid plants (generally referred to as anther culture).

Pollination. It consists of pollen grains reaching stigma of a flower.

Polycross. Open-pollination (assumed to be random mating) in isolation among a number of selected genotypes.

Polygenes. Genes with small and cumulative effect ; their expression is greatly affected by environment. They govern quantitative traits.

Polygenic Inheritance. Inheritance of quantitative characters due to the action of polygenes.

Polyploid. An individual with more than two identical or distinct genomes.

Population. *In Genetics,* all the individuals mating at random. *In statistics,* infinitely large number of observations of the same type.

Population Cross. A cross between two open-pollinated varieties (*Syn.*, varietal cross).

Population Improvement. Improvement of random mating populations through a scheme of selection with or without progeny test. It is essential to keep inbreeding to a low level.

Prepotency. Ability of an individual to produce progeny which are similar to each other and to itself.

Probability. The proportion of times an event may be expected to occur if an infinitely large number of trials are made.

Progeny Test. Evaluation of the genotypic value of an individual on the basis of the performance of its progeny.

Prapagule. Plant part used for propagation.

Protandry. Dehiscence of anthers before the stigma of the flower has become receptive.

Protogyny. Maturition of stigma (becoming receptive) before the dehiscence of anthers of that flower.

Pureline. Progeny of a single homozygous self-pollinated plant.

Pureline Selection. Isolation of purelines from a mixture nf purelines (*Syn.*, individual plant selection).

Quadriallel Analysis. Analysis based on double crosses obtained by crossing *n* homozygous lines in a diallel fashion, and then crossing the F_1's so generated according to a diallel scheme with the restriction that in any double cross a homozygous line must not occur more than once as a parent.

Quadrivalent. Structure produced by pairing among four homologous chromosomes during meiosis.

Qualitative Character. Character showing distinct classes and little or no effect of environment.

Quantitative Character. Character showing continuous variation and considerable effect of environment.

Quarantine. Isolation of an organism for observation on weeds, diseases and pests and for preventing their spread.

Random. An event whose occurrence is determined solely by chance and there is no discrimination.

Random Drift. See *Drift.*

Random Mating. A system of mating in which an individual has equal chance of mating with every other individual of the same population.

Range. The difference between the highest and the lowest values of observations in a sample.

Real Value of Seed. The percentage (by weight) of the seed sample which would produce seedlings of the variety under certification.

Recessive. An allele unable to express itself in heterozygous state.

Reciprocal Cross. Cross in which the line previously used as male is used as a female, while that previously used as female is used as male.

Recipient Parent. In backcross breeding, the parent to which one or few genes from the donor parent are transferred (*Syn.* recurrent parent).

Recombination. Production of new combinations of genes as a result of independent segregation of nonalleles or crossing over between linked genes ; usually the latter.

Recurrent Parent. See *Recipient Parent.*

Recurrent Selection. In cross-pollinated populations ; schemes of selection (on the basis of phenotype or progeny test), followed by intermating (in all combinations) of the selected plants or their selfed progeny to produce the population for the next cycle of selection, More than one cycle of selection practised.

Registered seed. Progeny of foundation seed.

Release of A Variety. The recommendation by the Central or a State variety Release Committee of an improved strain of a crop species for commercial cultivation in the whole or a part of the country.

Reproduction. Production of a new generation of individuals (progeny) by sexual or asexual means.

Repulsion Phase. Linkage between the dominant allele of one gene with the recessive allele of another gene.

Roguing. Removal of off-type plants of the same species.

Resistance. Ability of a host strain to restrict or even prevent the production of disease symptoms by a pathogen ; due to a gene for resistance.

Resistance Breaking Biotype. The biotype of an insect pest which has developed the ability or adaptation to infest a resistant variety of the host.

Restorer Gene. A gene, usually dominant, that effectively overcomes the effect of male sterile cytoplasm on male fertility, *i.e.*, produces functional male gametes even in the presence of the male sterile cytoplasm.

S_1, S_2, S_3 *etc.* First, second, third etc. generations derived by selfing of a plant (S_0) from a random mating population.

Sample A set of random observations taken from a population.

Sampling Error. Deviation of a sample value from that of the population due to the small size of the sample.

Seed. Part of a plant used for raising a seed crop or a commercial crop.

Seed Test. A series of tests on purity, moisture content and germination of a seed sample to determine its quality.

Seed Certification. Seed certification consists of field inspection and seed tests to ensure genetic and physical purity and good germination of seed lots, and issuance of a certificate to that effect.

Segregating Generations. The F_2 and the subsequent generations obtained through continued selfing of a hybrid between two or more strains.

Segregation. Separation of alleles at the time of gamete formation so that each gamete receives only one of the two alleles of a gene.

Selection. Differential reproduction rates of different genotypes.

Self-Fertility. Ability to set seeds on self-pollination.

Self-Fertilization. Union of male and female gametes from the same individual.

Self-Incompatibility. Lack of seed set on self-pollination.

Self-pollination. See *autogamy*.

Short-Term Inbred. A line derived by one or a few generations of inbreeding. Such a line is not homozygous or even nearly homozygous.

Sibs. Individuals having both the parents common, but derived from different gametes *i.e.*, they are not identical twins. *Half-sibs* are individuals having one common parent.

Sib Mating. Mating between sibs.

Simple Correlation Coefficient. A measure of the relationship between two variables.

Single Cross. A cross between two lines, usually inbred lines.

Single Seed Descent Method. A method of advancing the generations ; one seed from each F_2 plant of an F_2 and the subsequent generations is bulked to raise the next generation.

Species. A unit of taxonomic classification. Members of a species are more like each other than those from different species, and do not show barriers in sexual reproduction (except, of course, in cases of self-incompatibility and male sterility etc).

Specific Combining Ability. Deviation in performance of a cross combination from that predicted on the basis of the general combining abilities of the parents involved in the cross.

Sporogenesis. Production of mega- and micro-spores from mega- and micro-spore mother cells, respectively (through meiosis).

Stability Analysis. An analysis to estimate the adoptability of a genotype ; *adaptability* is the ability of a genotype to produce a relatively narrow range of phenotypes in the different environments.

Standard Deviation. Square root of variance (see variance). It is a measure of spread (variation) of a sample or population. ($\sqrt{s^2}$)

Standard Error. Standard deviation of a sample of means estimated from a number of random samples drawn from a single population. It is estimated as standard deviation divided by square root of the number of obervations in a sample. ($\sqrt{s^2/N}$)

Siation Trial. The trial conducted by a breeder to asses the performance of strains evolved by him as compared to a standard check ; usually at one location. (Syn., Preliminary Yield Trial).

Stock Seed. The seed produced by a breeder of a strain which has been identified but has not yet been released. It will be known as *breeder seed* once the strain is released and notified.

Strain. A group of individuals similar in phenotype and often in genotype. A strain is known as a *variety* when released for commercial cultivation by a variety release committee.

Substitution Line. A line in which a pair of chromosomes has been replaced by a pair from another variety of the same species. Used in aneuploid analysis.

Syn_0. The parental lines of a synthetic variety.

Syn_1. The population derived by intermating of Syn_0 lines in all possible combinations. Also, by open-pollination among the Syn_0 lines (parental lines).

Syn_2, Syn_3 etc. Generations derived through random mating from Syn_1, Syn_2 etc. generations, respectively.

Synapsis. Pairing between homologous chromosomes during meiosis, more particularly during zygotene stage of Prophase I.

Synthetic Allopolyploid. Allopolyploid produced experimentally by man.

Synthetic Variety. In cross-pollinated species, a variety obtained by mating in all possible combinations a number of lines that combine well with each other; Syn_1 or, more generally, Syn_2 and later generations.

Telophase. The last stage in cell division after the chromosomes/chromatids reach the opposite poles and before the nuclear membrane reappears.

Test Cross. In genetics, cross between a hybrid and the homozygous recessive strain. *In plant breeding,* cross between a plant or line and a tester (the tester may be an inbred, hybrid, synthetic or open-pollinated variety).

Tetraploid. An individual having four identical or distinct genomes.

Tetrasomic. An individual having one pair of chromosomes in addition to the normal somatic chromosome complement of the species $(2n+2)$.

Three-Way Cross. A cross between a single cross and an inbred.

Tissue Culture. Cultivation of plant cells and tissues in vitro on artificial media.

Tolerance. Ability of a host to avoid or minimise loss in productivity although it has been infected by a pathogen.

Top Cross. See *Inbred-Variety Cross.*

Trait. See *Character.*

Transgressive Breeding. Breeding methods designed to utilize transgressive segregation.

Transgressive Segregation. Appearance of individuals in the progeny from a hybrid which exceed either of the two parents of the hybrid with respect to one or more characters.

Translocation. Incorporation of a chromosome segment in a different chromosome.

Triallel Analysis. A genetic analysis based on the all possible three-way crosses among n parents.

Triple Test Cross Analysis. A genetic analysis based on the progenies derived by crossing a sample of randomly selected F_2 plants with the parental F_1 and the two homozygous parents.

Triploid. An individual having three identical or distinct genomes.

Trisomic. An individual having one chromosome in addition to the normal diploid chromosome complement of the species $(2n+1)$.

Trivalent. Structure formed by pairing among three homologous chromosomes during meiosis.

Uniform Regional Trials. See Coordinated Varietal Trials.

Univalent. During meiosis ; an unpaired chromosome.

Variability. The amount of variation present among the members of a population or species ; may have reference to one or more characters, and at genotypic or phenotypic levels.

Variance. Average of the squares of deviations of the observations of a sample from the mean of the sample drawn from a population. $[\Sigma(x-\bar{x})^2/N]$

Variation. Differences among individuals belonging to a single species or to different species. Variation may be due to genotype, environment, or both genotype and environment.

Varietal Cross. See *Population Cross.*

Varietal Mixture. A population of a self-pollinated species constituted by mixing the seeds of two or more varieties in equal or some other proportion.

Variety. In plant breeding, a strain released for commercial cultivation by a variety release committee; *in botany,* a sub-division of species based on form, function or both.

Vegetative Reproduction. Production of new individuals from vegetative parts.

Vigorous Biotype. A biotype of an insect pest which attacks both resistant and susceptible varieties of the host more successfully than the other biotypes. (*cf,* Resistance Breaking Biotype).

Virulence. Ability of a pathogen strain (pathotype or physiological race) to attack a strain of the host.

Vertical Resistance. Resistance, usually immunity, towards a virulent race/pathotype ; the specific virulent race/pathotype produces susceptible response. Generally, controlled by oligogenes.

Wide Cross. Cross between two species of the same genus or of different genera (*Syn.,* Distant Cross).

World Collection. See *Gene Bank.*

x. See *Basic Number.*

X_1, X_2, X_3 *etc.* First, second, third etc. generation, respectively, after irradation (with X-rays or some other mutageneic radiation) obtained through selfing or clonal multiplication.

Xenia. Effect of the genotype of pollen grain on the phenotype of seed tissues (embryo and endosperm).

Zygote. The immediate product of fusion of male and female gametes.

Zygotene. A stage during Prophase I of meiosis during which homologous chromosomes pair with each other.

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